Short Communication

Stereospecific high-performance liquid chromatographic assay of metoprolol

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ABSTRACT

A valid, sensitive high-performance liquid chromatographic technique is reported for the separation of the two enantiomers of metoprolol in human plasma. The procedure involves pre-column derivatization with the homochiral reagent S-(+)-1-(1-naphthyl)-ethyl isocyanate. Once formed, the diastereomers are separated using normal-phase high-performance liquid chromatography. Fluorescence detection (220 nm excitation; no emission filter) was utilized, resulting in baseline resolution ($R_s > 1.5$). The peaks corresponding to metoprolol enantiomers were free from interference throughout the examined range of 5–500 ng/ml; accuracy and precision were within approximately 10%. Analysis of a plasma sample collected from a healthy volunteer demonstrated that the assay is applicable to clinical studies.

INTRODUCTION

Metoprolol (Fig. 1A) is a β_1-selective aryl oxypropanolamine adrenergic antagonist (β-blocker) used extensively in the treatment of a variety of cardiovascular disorders. As with most other β-blocking drugs, the S-(−)-enantiomer has significantly more β-adrenergic receptor affinity [1,2] than the R-(+)-enantiomer. In addition to the differing pharmacologic properties of β-blocking drug enantiomers, it is also known that some drugs within this class of compounds

may exhibit stereoselective pharmacokinetics [3–7].

To date, most reported analytical techniques for determination of metoprolol in biological samples have utilized non-stereospecific high-performance liquid chromatography (HPLC) [8–13]. In a review article by Davies [3], a number of procedures for β-blocker enantiomer separation were reported. In this article, several methods employed the use of pre-column derivatization with subsequent separation of diastereomers of metoprolol on achiral stationary phases. Additionally, the use of chiral stationary phases has also been reported [3,4]. Interestingly, in our experience, the use of isocyanates as derivatizing reagents with or without quenching agents often produced unwanted peaks that eluted sufficiently

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OCH₂CH(OH)CH₂NHCH(CH₃)₂

CH₂CH₂OCH₃

A

CH(CH₃)NCO

B

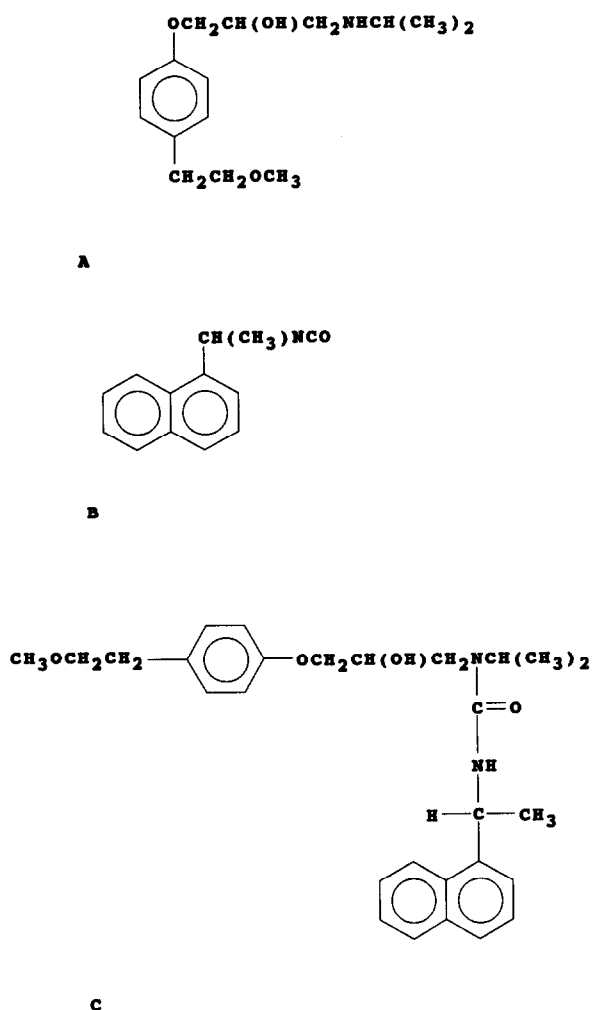CH₃OCH₂CH₂——⟨O⟩——OCH₂CH(OH)CH₂NCH(CH₃)₂

C=O

NH

H—C—CH₃

C

Fig. 1. Structures of (A) metoprolol, (B) NEIC, and (C) derivatized metoprolol.

late [14]. Processing large numbers of samples during clinical studies, therefore, was less efficient when utilizing reversed-phase HPLC. Consequently, normal-phase HPLC was necessary.

In this paper we report a sensitive, stereospecific normal-phase HPLC assay suitable for determination of metoprolol in human plasma.

EXPERIMENTAL

Chemicals

Racemic metoprolol tartrate was purchased

from Sigma (St. Louis, MO, USA). Internal standard (I.S., (±)-propranolol hydrochloride) was purchased from Aldrich (Milwaukee, WI, USA). Pure enantiomers of metoprolol were obtained as gifts from Dr. R. Mehvar (Drake University, Des Moines, IA, USA). The derivatizing reagent S-(+)-1-(1-naphthyl)ethyl isocyanate (NEIC, Fig. 1B) was obtained from Aldrich. Other chemicals including hexane, acetonitrile, chloroform, methanol, sodium hydroxide and water (BDH, Toronto, Canada) were analytical grade.

Chromatography

Samples were vortex-mixed with a Genie 2 mixer (Fisher Scientific, Edmonton, Canada) and centrifuged with a Dynac II centrifuge (Becton-Dickinson, Parsippany, NJ, USA). Evaporation of solvents utilized a Model SC 100 Savant Speed Vac concentrator–evaporator (Emerston Instruments, Scarborough, Canada). The HPLC system consisted of a Waters Model 590 pump and a Waters Model 712 Wisp autosampler (Mississauga, Canada). Detection of the diastereomers utilized an Applied Biosystems Model 980 fluorescence detector (Technical Marketing, Edmonton, Canada) which was set at 220 nm for excitation with no emission filter. Peaks were quantitated using a Model 745B Waters integrator. Analytical separation was accomplished on silica particles using a 25-cm stainless-steel column (Whatman, Clifton, NJ, USA) with 5 μm particle size.

The mobile phase consisted of hexane–chloroform–methanol (85:14:1, v/v) pumped at a flow-rate of 2.0 ml/min. Sample preparation and analysis were conducted at room temperature.

Standard solutions

A 50 μg/ml (as base) stock solution of each metoprolol enantiomer was prepared in water (solution 1). The I.S. was prepared as 5 μg/ml (as base) of each enantiomer in water (solution 2). A second solution of metoprolol (solution 3) was prepared as triethylamine in chloroform (0.04 μmol/l) to give a final concentration of 50 μg/ml (as base). The NEIC solution was prepared in chloroform as a 0.05% (v/v) solution.

Sample preparation

To 1.0 ml of drug-free human plasma was added metoprolol (solution 1) to yield final concentrations of 5, 10, 25, 50, 100, 250 and 500 ng/ml of each enantiomer. After addition of 5 μg of I.S. (solution 2) and 50 μl of 0.1 M sodium hydroxide, 4 ml of chloroform were added and the resultant mixture was vortex-mixed for 30 s and centrifuged at 1800 g for 5 min. The upper aqueous layer was removed by vacuum, thus leaving the organic layer. This layer was then evaporated to dryness using the Speed Vac concentrator–evaporator. To the resulting residue was added 200 μl of the NEIC solution. The solution was again vortex-mixed for 30 s and aliquots ranging from 75 to 150 μl were injected on to the HPLC column.

Extraction yield

From solution 3 were added 10, 100 or 500 ng/ml metoprolol enantiomers ($n = 3$ of each concentration) into clean, dry glass tubes after the appropriate dilution using chloroform. The solutions were evaporated to dryness and 1.0 ml of plasma was added to each tube. The metoprolol enantiomers were extracted as previously described, with the exception that exactly 2.0 ml of the extract was transferred to a clean glass tube and derivatized. To compare these extracted samples with those that were not extracted, an equivalent amount of metoprolol (from solution 3) was added to another set of glass tubes ($n = 3$ of each concentration), immediately evaporated to dryness and derivatized. The peak areas of metoprolol extracted from plasma versus unextracted equivalent metoprolol were compared under identical chromatographic conditions.

Derivatization yield

Using solution 3, metoprolol enantiomer concentrations of 10, 100 or 500 ng/ml ($n = 6$ of each concentration) were added to glass tubes and evaporated to dryness after the appropriate dilution with chloroform. Each sample was derivatized as previously described and a non-stereospecific chromatographic method was used to quantitate the underivatized metoprolol remaining. A UV–VIS Model PU 8740 scanning spectrophotometer (Philips Electronic, Cambridge, UK) was used to detect any underivatized metoprolol. A Model BD80 Kipp & Zonen plotter was used to record the spectra (Netherlands).
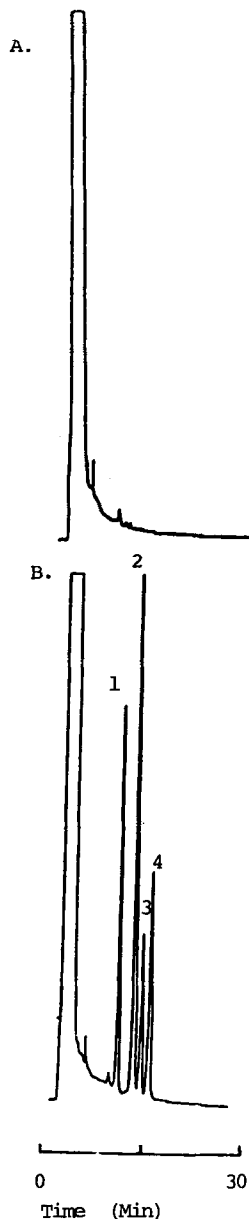

Fig. 2. Chromatograms of (A) blank plasma and (B) plasma spiked with 100 ng/ml of each metoprolol enantiomer. Peaks: 1 = R-(+)-I.S.; 2 = S-(−)-I.S.; 3 = R-(+)-metoprolol; 4 = S-(−)-metoprolol.

RESULTS AND DISCUSSION

There have been numerous reports of stereo-specific assays for β-blocking drug determination utilizing homochiral derivatizing reagents [3,15–17]. Our laboratory has reported the use of NEIC as a derivatizing reagent for the analysis of other chiral drugs [14,18–20]. Although others have also reported the use of ioscyanates including S-(−)-phenylethyl isocyanate for the analysis of metoprolol, a more lengthy derivatization time was required during sample preparation [16].

Separation of metoprolol enantiomers was achieved using optically pure NEIC, which formed diastereomers that were subsequently chromatographed using normal-phase HPLC. Using this method, individual enantiomers of metoprolol were measured in human plasma. The reaction of NEIC with metoprolol enantiomers will result in the formation of a urea derivative (Fig. 1C). The formation of this derivative has been confirmed by others utilizing iso-cyanates as derivatizing reagents with β-blocking drugs [14,17]. Fig. 2 depicts a representative chromatogram illustrating baseline resolution of the peaks corresponding to the diastereomers (R_s > 1.5). The peaks corresponding to the metoprolol enantiomers eluted at approximately 14.1 and 16.2 min. The first- and second-eluting metoprolol peaks corresponded to R-(+)-metoprolol and

S-(−)-metoprolol, respectively, as confirmed by chromatographing the pure enantiomers. The R- and S-I.S. eluted at approximately 8.5 and 10.5 min, respectively, as confirmed by chromatography of the pure enantiomers of propranolol. Consequently, the total run time for the assay was less than 20 min.

Although it is known that metoprolol is metabolized [8], we were unable to confirm the detection of metabolites at present, as suitable laboratory reference standards were not available. Calibration curves for R-metoprolol and S-metoprolol were typically described by $y = 0.012 + 0.0088x$ and $y = -0.016 + 0.0080x$, respectively, where y corresponds to the peak-area ratio of drug to I.S. and x to enantiomer concentration. Excellent linearity was observed for all calibration curves ($r^2 > 0.997$).

The results in Table I describe the accuracy (analytical recovery, AR) and precision (coefficient of variation, C.V.). Both accuracy and precision values throughout the concentration range of 5–500 ng/ml were within approximately 10% of the expected values.

The derivatization yield was not less than 95%, as calculated by comparing the UV spectra of undervatized metoprolol with the spectra of underivatized metoprolol remaining after derivatization with NEIC.

The extraction yield of metoprolol from plas-

TABLE I

ACCURACY AND PRECISION OF THE METHOD

$n = 9$ (three sets for three days).

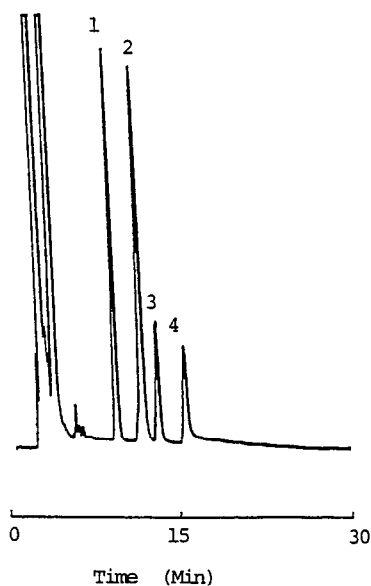| Concentration added (ng/ml)	Measured concentration (mean ± S.D.) (ng/ml)		Accuracy (%)		Precision (C.V. %)	
	R	S	R	S	R	S
5	5.5 ± 0.6	5.5 ± 0.5	110	110	10.7	10.7
10	10 ± 1	9.9 ± 0.6	100	99	10.0	6.4
25	24 ± 3	24 ± 2	96	96	10.0	9.2
50	52 ± 3	50 ± 5	104	100	5.6	8.7
100	103 ± 5	103 ± 6	103	103	5.3	6.3
250	243 ± 11	242 ± 14	97	96	4.7	6.0
500	505 ± 5	505 ± 6	101	101	0.9	1.2

Fig. 3. Chromatogram of a plasma sample taken 12 h following oral administration of a single 100-mg tablet of metoprolol to a healthy volunteer. For peak identification, see Fig. 2.

ma, as indicated by comparing extracted *versus* unextracted samples, was 92.0 ± 11.60% for *R*-metoprolol and 90.24 ± 18.73% for *S*-metoprolol. The reaction of *R* and *S*-metoprolol enantiomers with NEIC was deemed complete within 2 min at room temperature under anhydrous conditions. Sample integrity did not appear to be changed when samples were stored for up to 48 h at room temperature. This was confirmed by repeatedly injecting the same sample onto the HPLC for up to 48 h.

Fig. 3 represents a chromatogram of plasma taken from a healthy subject after administration of 100 mg of metoprolol. This method, therefore, is applicable to the analysis of plasma samples containing metoprolol in individuals administered such.

In conclusion, a sensitive and stereospecific normal-phase HPLC analysis of metoprolol was reported, using optically pure NEIC as derivatizing reagent. The reaction of NEIC with metoprolol proceeded rapidly at room temperature. The assay is valid for determination of metoprolol in clinical samples with minimal sample preparation

and a run time that is suitable for processing numerous samples on a daily basis. Furthermore, the absence of any late-eluting peaks during sample runs may suggest that normal-phase HPLC offers a distinct advantage over reversed-phase when using isocyanates.

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