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Letter to the Editor

High-performance liquid chromatographic determination of diacetolol enantiomers

Sir,

A number of recent publications [1–5] have addressed the importance of separation, identification and quantification of chiral drugs. Subsequently, a substantial amount of literature has surfaced regarding the pharmacokinetics of an important class of chiral drugs, the β-blocking drugs. One such drug, acebutolol (AC), is a cardioselective β-blocker marketed as the racemic mixture, where only the (−)-isomer has β-blocking activity [6]. Upon first pass through the liver, AC is extensively metabolized to an active (equipotent) chiral metabolite, diacetolol (DC). Analytical techniques, therefore, must measure concentrations of AC and DC enantiomers to be useful Although several methods have been employed to measure AC and DC [7–11], most methods were non-stereospecific. Sankey *et al.* [6] separated AC and DC enantiomers with the use of the chiral derivitizing reagent, *S*(−)-N-trifluoroacetylprolylchloride (TPC). However, commercial TPC is contaminated with 4 to 15% of the *R*(+)-enantiomer and also racemizes upon storage [12]. Gulaid and Houghton [12] recently reported a stereospecific method utilizing *R*(+)-1-phenylethyl isocyanate. However, the lower limit of detection (0 05 μg/ml) limited its application in pharmacokinetic studies. We recently reported a convenient and sensitive stereospecific high-performance liquid chromatographic (HPLC) assay for AC [13]. We have now determined that this assay is suitable for measuring concentrations of DC enantiomers in plasma and urine. In this communication we report an HPLC method suitable for the simultaneous separation of the enantiomers of AC and DC.

EXPERIMENTAL

Chemicals and chromatography

Racemic DC and *R*(+)-DC (Rhone-Poulenc, Dagenham, U.K.) and racemic pindolol (internal standard, I.S., Sandoz, Dorval, Canada) were obtained as gifts The derivatizing reagent, *S*(+)-1-(naphthyl)ethyl isocyanate (NEIC) and all other chemicals were as previously described for the acebutolol analysis [13]. The HPLC system has been described previously [13].

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Standard solutions and sample preparation

A stock solution of DC (as hydrochloride salt) was prepared in water to a final concentration of 50 mg/l of the base. A second solution of the same concentration was prepared as 0.01% (v/v) triethylamine in chloroform for the determination of extraction and derivatization. An I.S. solution was prepared to a final concentration of 500 mg/l in methanol. All solutions were kept refrigerated at 4°C Blank plasma and urine samples were spiked with DC to yield final enantiomer concentrations of 10, 25, 50, 100, 250 and 500 ng/ml and 0.1, 0.25, 0.5. 0.1, 2.5 and 5.0 mg/l, respectively. A solution of 0.1% (v/v) NEIC was prepared in chloroform and stored under nitrogen at −20°C. Samples were prepared as previously described for acebutolol [13].

Extraction yields

To 1 ml of either plasma or urine were added 50 or 500 ng of DC After the samples were extracted, derivatized and chromatographed, the peak areas of DC diastereoisomers were compared with identical concentrations of DC enantiomers in chloroform which were directly derivatized (unextracted) with 0.1% (v/v) NEIC. The percentage recovery was calculated from the peak areas of extracted *versus* unextracted DC enantiomers.

RESULTS AND DISCUSSION

This HPLC method which employed fluorescence detection after chiral derivatization with NEIC offered a sensitive and convenient technique for measurement of DC enantiomers. Under the chromatographic conditions stated, virtual baseline separation of DC diastereoisomer derivatives was achieved (resolution, $R > 1.45$). The retention times for *R*- and *S*-DC were approximately 23 and 25 min, respectively, and were free from any interfering peaks (Fig. 1). The exact order of elution was determined by derivatization and chromatography of the pure *R*-enantiomer of DC. Under these conditions, peaks corresponding to I.S. (order of elution of enantiomers not confirmed) and *R* and *S*-AC enantiomers eluted at approximately 4.5, 5, 8, and 9 min, respectively (Fig. 1) For the sake of consistency, only the first eluting I.S. peak was used for quantification of DC enantiomers.

The calibration curves for *R*- and *S*-DC/I.S. peak-area ratios displayed excellent linearity in both plasma and urine ($r^2 \geq 0.995$). Typical calibration curves obtained for *R*- and *S*-DC in plasma were described by $y = 0.0085x + 0\ 0496$ and $y = 0.0085x + 0.0427$, respectively, where y was the peak-area ratio (DC/I.S.) and x was the enantiomer concentration. The minimum quantifiable concentration (signal-to-noise ratio of 4:1) was 1 ng/ml.

The intra- and inter-day reproducibility was determined by preparing three sets of calibration curves daily, on three separate days. Good reproducibility was observed over the entire concentration range (coefficient of variation $< 10\%$). Table I summarizes the accuracy and precision of the method.

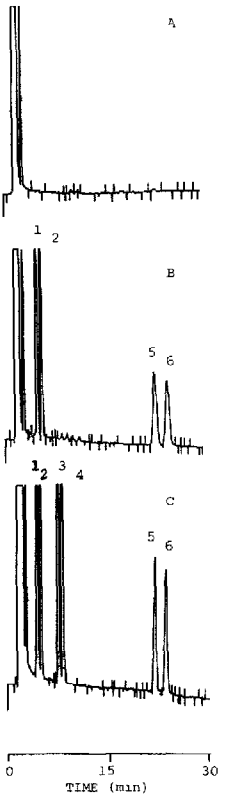

Fig. 1 Chromatograms of (A) blank plasma, (B) 50 ng/ml of each enantiomer of DC added to drug-free plasma and (C) 50 ng/ml of each enantiomer of both AC and DC added to drug-free plasma. Peaks 1 and 2 = I.S. (order of enantiomer elution not confirmed); 3 and 4 = *R*- and *S*-AC, 5 and 6 = *R*- and *S*-DC.

TABLE I

ACCURACY AND PRECISION OF THE METHOD (n = 9)

Plasma concentration (ng/ml)			Error (%)		C.V. (%)	
Added	Measured (mean ± S.D.)		*R*	*S*	*R*	*S*
	R	*S*				
10.0	10.9 ± 0.9	10.9 ± 0.3	+9.2	+9.7	8.2	2.6
25.0	24.8 ± 1.8	25.2 ± 1.6	−0.9	+0.8	7.1	6.3
50.0	53.5 ± 3.9	52.7 ± 5.0	+7.0	+5.4	7.3	9.5
100.0	101 ± 6.7	101 ± 6.7	+0.6	+1.0	6.7	6.6
250.0	260 ± 11	261 ± 13	+3.9	+4.5	4.4	4.8
500.0	498 ± 3.6	497 ± 4.4	−0.5	−0.5	0.7	0.9

The extraction yields of *R*- and *S*-DC from plasma were 26.0 ± 3.0 and 28.4 ± 3.4% at 50 and 500 ng/ml, respectively (n = 6). Recovery from urine was determined to be 30.1 ± 2.8% at 0.5 mg/l and 29.0 ± 3.8% at 5.0 mg/l (n = 6) Improvement of the extraction was not necessary, as. (1) extraction of AC was greater than 80% under these conditions [13] and (2) the required sensitivity for DC enantiomer detection was adequate for conducting clinical studies.

DC reacted with NEIC to form the urea diastereoisomer derivative, and not the carbamate. This reaction has been confirmed by others using mass spectrometric analysis [13,14]. The derivatization reaction time was measured by comparing the peak areas at 5, 15, 30 and 60 min after addition of NEIC to DC. The reaction proceeded quickly, as increases in peak areas of DC were not observed after 5 min. Studies using isocyanates to derivatize structurally similar compounds have also reported rapid derivatization [15,16]. Additionally, once formed, the DC diastereoisomers were stable at room temperature in chloroform for at least 72 h.

In conclusion, this normal-phase HPLC method is convenient for the determination of the enantiomers of DC, the main (active) metabolite of AC. This assay procedure is convenient and sensitive and can be applied to metabolic studies of subjects administered AC

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