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Note

Separation of acebutolol and diacetolol diastereomers by reversed-phase high-performance liquid chromatography

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Acebutolol [*RS*-(±)-1-acetyl-4-butyramidophenoxyl-2-hydroxy-3-isopropyl-aminopropane] is an important β-adrenoceptor antagonist which is available as a racemic mixture of two optical isomers.

Meffin and co-workers[1–3] and Gulaid *et al.*[4], employing specific methods, have demonstrated the extensive metabolism of acebutolol to diacetolol (Fig. 1). Acebutolol and diacetolol have chiral centres in the hydroxyisopropoylaminopropane side chain. The *S*-(−)-enantiomers of this class of drugs generally exhibit greater cardiac β-adrenoceptor blocking activity[5,6], while *R*-(+)-enantiomers have a membrane stabilizing effect[7]. This is in contrast to thalidomide in which, after i.p. administration, only the *S*-(−)-enantiomer was shown to be teratogenic in SWS mice and Natal rats[8].

The progress in asymmetric synthesis of chiral compounds in recent years[9] has stimulated the development of new analytical techniques for evaluating enantioselectivity and stereoselectivity of their disposition. In the case of a racemic drug, where only one of the enantiomers is pharmacologically active, it is important that pharmacokinetic and metabolism studies be directed to the active species.

The most frequently used method is the derivatization of the drug with a chiral reagent, followed by chromatographic resolution of the resulting diastereomers.

The enantiomers of acebutolol and diacetolol have been resolved by high-performance liquid chromatography (HPLC) as the derivative formed with *S*-(−)-N-trifluoroacetyl prolylchloride (TPC)[10]. However, commercial TPC is contaminated with 4% to 15% of the (+)-enantiomer and the reagent rapidly racemizes during storage. *S*-(−)-1-Phenylethyl isocyanate (PEIC)[11] has been used for the resolution of propranolol. It was found to be more stable than TPC. In this communication, we describe an HPLC method for the resolution of enantiomers of acebutolol, diacetolol and the internal standard (Fig. 1) in plasma by forming their diastereomers using *R*-(+)-1-phenylethyl isocyanate.

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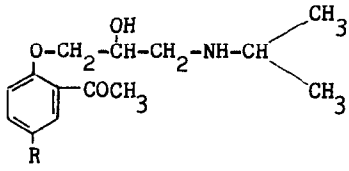

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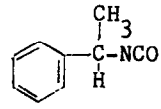

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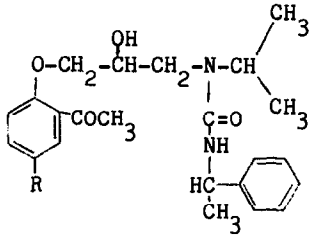
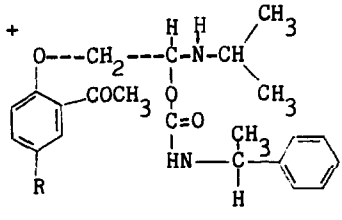


Urea derivative



Carbamate derivative

	R
Acebutolol	-NH-CO $(CH_2)_2CH_3$
Diacetolol	-NH-$COCH_3$
M&B 17,764	-NH-$COCH_2$ CH_3

Fig. 1. Structures of acebutolol, diacetolol and M&B 17,764 and the possible urea and carbamate derivatives when reacted with *R*-(+)-1-phenyl ethyl isocyanate.

EXPERIMENTAL

Chemicals and reagents

(±)-Acebutolol, (±)-diacetolol, (±)-internal standard, (−)-acebutolol, (+)-acebutolol, (−)-diacetolol and (+)-diacetolol were all synthesised at May & Baker, Dagenham, U.K. *R*-(+)- and *S*-(−)-1-phenylethyl isocyanate were purchased from Aldrich, Gillingham, U.K. Diethyl ether and methanol (HPLC grade) were from Rathburn (Walkerburn, U.K.). Sodium hydroxide was from May & Baker.

Chromatography

The HPLC system consisted of Waters auto-injector WISP 710B, M-6000 pump (Waters Assoc., Hartford, U.K.), Perkin-Elmer 3000 fluorescence spectrometer (Perkin-Elmer, Beaconsfield, U.K.) and Philips PM 8252 dual-pen recorder (Pye-Unicam, Cambridge, U.K.). The stationary phase was ODS2 (5 μm) packed in a 250 × 4.6 mm I.D. column (Phase-Separations, Queensferry, U.K.). Analyses were performed using a mobile phase of water–methanol–triethylamine (50:50:0.05) at a flow-rate of 1.2 ml/min at a pressure of 4000 p.s.i. and at room temperature (*ca.* 21°C).

Extraction and derivatization of S-(−)- and R-(+)-acebutolol and diacetolol

Sodium hydroxide (1 *N*, 0.5 ml) was added to plasma (1 ml) containing ace-

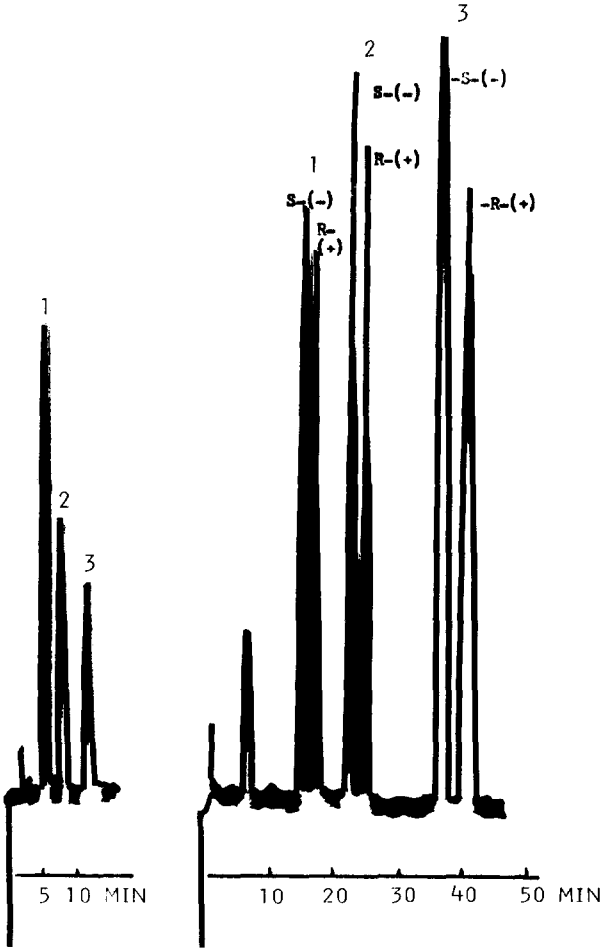


Fig. 2. High-performance liquid chromatograms of (left) diacetolol, M&B 17,764 and acebutolol racemates and (right) dog plasma extracts of diacetolol, M&B 17,764 and acebutolol diastereomers. Column: 250 × 4.6 mm I.D. ODS2 (5 μm); mobile phase: water–methanol–triethylamine (50:50:0.05); flow-rate: 1.2 ml/min; pressure: 4000 p.s.i.; fluorescence detection: excitation wavelength 238 nm, emission wavelength 450 nm. Peaks: Left: 1 = diacetolol; 2 = M&B 17,764; 3 = acebutolol; right: *S*-(−)- and *R*-(+)-1-phenylethyl isocyanate derivatives of (1) diacetolol, (2) M&B 17,764 and (3) acebutolol.

butolol, diacetolol and the internal standard (M&B 17,764, see Fig. 1). The sample was then extracted with diethyl ether (5 ml). The tubes were shaken for 5 min and then centrifuged at 450 *g*. The ether layer was collected and evaporated at 50°C in a stream of oxygen-free nitrogen. The residue was dissolved in dry dichloromethane (100 μl). *R*-(+)-1-Phenylethyl isocyanate in dichloromethane (20 μl of 100 μg/μl) was added and reacted for 30 min at room temperature. Diethyl ether (100 μl) was added to the tubes, shaken for 1 min and then evaporated to dryness at 50°C under oxygen-free nitrogen. The samples were dissolved in 100 μl of mobile phase and injected onto the HPLC column.

RESULTS AND DISCUSSION

R-(+)-1-Phenylethyl isocyanate reacted rapidly with acebutolol, diacetolol and the internal standard (M&B 17,764) at room temperature forming the urea derivatives (Fig. 1). Addition of excess of the isocyanate reagent (up to 1000-fold) did not make any difference to the urea derivatives and did not form any carbamate. The formation of the urea derivative was confirmed by IR, UV, NMR and mass spectroscopy. Under the chromatographic conditions used all three racemates were separated into their respective diastereomers (Fig. 2). For both acebutolol and diacetolol the *S*-(−) isomer eluted before the *R*-(+) isomer, and it is reasonable to assume that this would also occur with M&B 17,764. Acebutolol and diacetolol racemates and their diastereomers all produced linear calibration graphs.

The range covered was 0.05–15 μg/ml plasma. Correlation coefficients were all better than 0.995 with a lower limit of detection of *ca.* 0.05 μg/ml. The extraction efficiency for both acebutolol and diacetolol was *ca.* 65–75% with a coefficient of variation less than 5% at 0.5 μg/ml plasma. Typical chromatograms are shown in Fig. 2.

The current assay was applied to assess the pharmacokinetics of separated *S*-(−)- and *R*-(+)-acebutolol and *S*-(−)- and *R*-(+)-diacetolol post-dose plasma concentrations in man and dog (results to be reported elsewhere).

In conclusion, a new stereospecific and sensitive HPLC method capable of separating the *S*-(−) and *R*-(+) enantiomers of acebutolol and its major metabolite, diacetolol, in human and dog plasma is described.

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