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Note

High-performance liquid chromatographic assay of dilevalol, a β-adrenoceptor blocker, in rat and human plasma

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Dilevalol, the *R,R* optical isomer of labetalol, is a new antihypertensive drug that combines β-receptor blockade with vasodilating properties due to β_2-agonism [1,2].

Several high-performance liquid chromatographic (HPLC) procedures for determination of labetalol (or dilevalol) using spectrophotometric and fluorimetric detection have been described [3–8]. With 2 ml of plasma and UV detection, a limit of quantitation of 40 ng/ml was reported [3]. Two more sensitive HPLC procedures (10 ng/ml) were also described [4,5]. Use of fluorimetric detection led to improved sensitivity. An HPLC procedure involving a post-column alkalinization to optimize the fluorescent yield of labetalol was described [6]: with 1.0 ml of plasma the limit of quantitation was 8.0 ng/ml. An interesting sorbent, PRP-1, polystyrene–divinylbenzene resin, was used for determination of labetalol [7]. With 0.5 ml of plasma the limit of quantitation was 4 ng/ml. A significant modification of this method improved the limit of detection for dilevalol to 1 ng/ml [8]. This paper describes a simple procedure for the determination of dilevalol in rat and human plasma using UV detection.

EXPERIMENTAL

Materials and reagents

Dilevalol · HCl and ICI 118 551, *erythro*-(±)-1-(7-methylindan-4-yloxy)-3-isopropylaminobutan-2-ol (I.S.), were supplied by Schering-Plough (Bloomfield, NJ, U.S.A.) and ICI (Macclesfield, U.K.), respectively. Acetonitrile, "LiChrosolv", and all other chemicals of reagent grade were obtained from Merck (Darmstadt, F.R.G.). Demineralized water was used throughout. Stock solutions of dilevalol and I.S. in water stored at 4°C were stable for at least one month. Working plasma standards were stored at −20°C and were stable for at least two months. All drug concentrations were calculated as free base.

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HPLC equipment and chromatographic conditions

The chromatographic system consisted of a Series 410 LC pump, a Model LC-95 UV–VIS variable-wavelength detector and an LCI-100 laboratory computing integrator (Perkin-Elmer, Norwalk, CT, U.S.A.). A prepacked μBondapak C_{18} column (10 μm particle size, 300 mm × 3.9 mm I.D., Waters Assoc., Milford, MA, U.S.A.) preceded by a LiChroCART RP 18 guard column (10 μm particle size, 25 mm × 4 mm I.D., Merck) were used for the chromatographic separation. The samples were injected with a Model 7125 valve (Rheodyne, Cotati, CA, U.S.A.) equipped with a 150-μl loop. The mobile phase consisted of 45 m*M* KH_2PO_4 and 25% (v/v) acetonitrile. The pH was adjusted to 3.0 by addition of 85% H_3PO_4 solution, and the mobile phase was filtered through a 0.5-μm filter (Millipore, Bedford, MA, U.S.A.) and degassed by helium bubbling. The flow-rate was maintained at 2.0 ml/min at a pressure of 18.1 MPa. Chromatographic analysis was performed at room temperature, and the effluent was monitored at 216 nm.

Extraction procedure and HPLC separation

Plasma (0.5 ml) was dispensed into a glass tube fitted with a PTFE screw cap. After the addition of 25 μl of I.S. (1 mg/100 ml), 0.5 ml of 0.05 *M* Tris buffer (pH 9.0) and 4.0 ml of diethyl ether, the sample was shaken for 10 min. The tube was centrifuged at 2000 *g* for 5 min. The aqueous layer was frozen in a dry-ice–acetone bath, and the organic layer was transferred to a clean glass tube containing 200 μl of 0.05 *M* sulphuric acid. Dilevalol and I.S. were back-extracted into the acidic layer by shaking for 10 min, and the layers were again separated as above. The organic layer was discarded and the glass tube was placed in a water-bath at 45°C for 15 min to evaporate the remaining diethyl ether. A 150-μl aliquot of the acidic extract was injected into the chromatographic column. Dilevalol and I.S. had retention times of 5.5 and 14.3 min, respectively. Rat and human plasma contained few endogenous peaks, which did not interfere with the analysis.

RESULTS

Linearity, precision and accuracy

Rat plasma samples spiked at six different concentrations of divelalol were analysed. The peak-height ratios (dilevalol to I.S.) were linearly related to dilevalol concentration over the range 12.5–400 ng/ml. The equation for the straight line was $y = 0.0147 + 0.0085x$, with $r = 0.99$. Over the range 12.5–400 ng/ml, relative errors (R.E.) ranged from 0.5 to 3.1% (mean 1.4%); the precision had a coefficient of variation (C.V.) ranging from 1.5 to 9.5% (mean 4.5%). Human plasma samples, spiked with different dilevalol concentrations, were also analysed. No differences in chromatographic separation and quantitation were observed between human and rat plasma.

Limit of quantitation and recovery

The limit of dilevalol quantitation determined in rat plasma was 12.5 ng/ml; the accuracy (R.E. 3.1%) and precision (C.V. 9.5%) being quite good. Figs. 1 and 2 show chromatograms obtained from rat and human plasma. From plasma samples containing 50.0 and 400 ng/ml dilevalol the analytical recovery was 93% (S.D. = 3%; $n = 6$) and 86% (S.D. = 3%; $n = 6$), respectively. The recovery of I.S. was 95% (S.D. = 6%; $n = 6$).

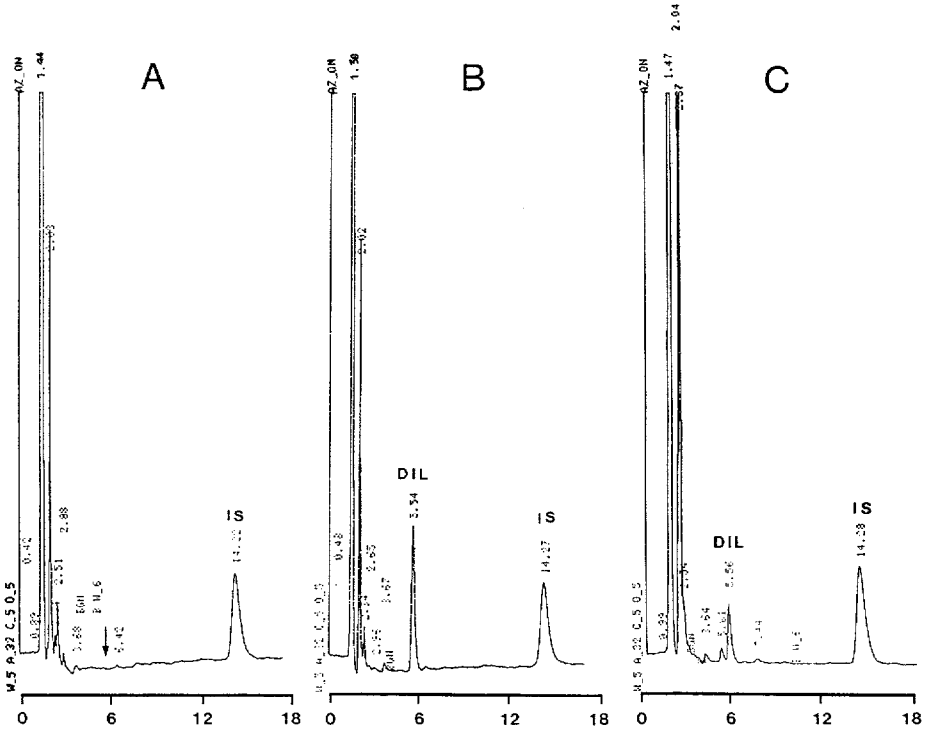

Fig. 1. (A) Chromatogram obtained from 0.5 ml of rat plasma with internal standard. (B) Chromatogram obtained from 0.5 ml of rat plasma with internal standard and spiked to a dilevalol concentration of 200 ng/ml. (C) Chromatogram obtained from 0.5 ml of rat plasma taken 30 min after oral administration of dilevalol (30 mg/kg). The concentration is found to be 70.8 ng/ml. An unknown peak with a retention time of 5.0 min appears before the dilevalol peak. Attenuation, 0.032 a.u.f.s. Peaks: IS = internal standard; DIL = dilevalol.

Stability and day-to-day reproducibility

Rat plasma samples containing 50.0 and 400 ng/ml dilevalol stored at −20°C were analysed in duplicate on days 1, 2, 3, 15, 17, 20 and 60. Mean values of dilevalol concentration were 49.73 ng/ml (C.V. = 4.2%, R.E. = 0.5%) and 394.72 ng/ml (C.V. = 2.0%, R.E. = 1.3%), respectively.

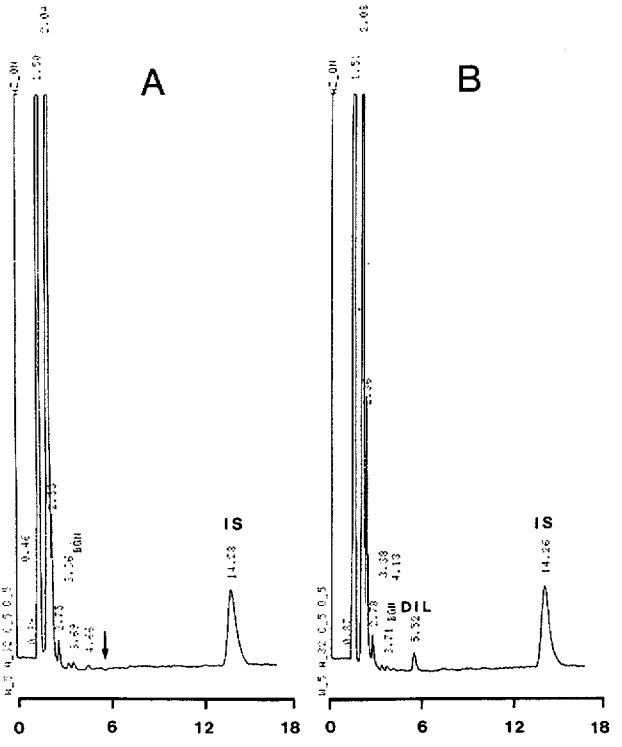

Fig. 2. (A) Chromatogram obtained from 0.5 ml of human plasma with internal standard. (B) Chromatogram obtained from 0.5 ml of human plasma with internal standard and spiked to a dilevalol concentration of 25.0 ng/ml. Peaks: IS = internal standard; DIL = dilevalol.

CONCLUSION

In this paper a simple HPLC method for determination of dilevalol (and labetalol) using UV detection has been validated for plasma concentrations ranging from 12.5 to 400 ng/ml. This HPLC method, developed for the determination of dilevalol in animal studies, could be employed to analyse human samples. With UV detection, the sensitivity is less than that with fluorimetric detection, but the limit of quantitation (12.5 ng/ml) is sufficient to carry out routine clinical monitoring.

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