Journal of Chromatography, 175 (1979) 208–210

CHROM. 11,862

Note

Determination of labetalol in human plasma by high-performance liquid chromatography

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(Received March 9th, 1979)

Labetalol (2-hydroxy-5-[1-hydroxy-2-(1-methyl-3-phenylpropylamino)ethyl] benzamide hydrochloride) is a combined α- and β-adrenoceptor antagonist which is effective in the treatment of hypertension[1]. A recent study has shown that there is a clear relationship between the dose of labetalol administered and the plasma concentration[2]. Although the daily dose can vary from 200 to 2400 mg the stated effective plasma level is expected to be greater than 0.1 μg/ml.

The method of analysis used in the above study[2] was fluorimetry since derivatives of labetalol employed for gas–liquid chromatography were unsuitable for quantitative estimation of the drug. A high-performance liquid chromatography (HPLC) method is described in this paper which is rapid and sensitive allowing the estimation of the drug down to a level of 0.04 μg/ml.

EXPERIMENTAL

Reagents

Redistilled diethyl ether; buffer solution pH 9.0: saturated ammonium chloride solution adjusted to pH 9.0 with concentrated ammonium hydroxide; acetonitrile (Nanograde; Mallinckrodt, St. Louis, Mo., U.S.A.).

All glassware was silylated with Dri-film SC-87 (Pierce, Rockford, Ill., U.S.A.). The internal standard was pericyazine at a concentration of 0.2 μg/μl in ethanol.

Apparatus

A Waters Assoc. high-performance liquid chromatograph equipped with a Waters 450 variable-wavelength UV detector operated at 233 nm was used. The column (30 cm × 4 mm) was packed with μBondapak C_{18} (Waters Assoc., Milford, Mass., U.S.A.). Samples were introduced by means of a variable-loop injector (Waters Model U6K). The eluent was 35% acetonitrile in 45 m*M* KH_2PO_4 adjusted to pH 3.0 with phosphoric acid, used at a flow-rate of 1.0 ml/min. Under these conditions the elution time of labetalol and the internal standard was 3.9 and 7.3 min, respectively (Fig. 1).

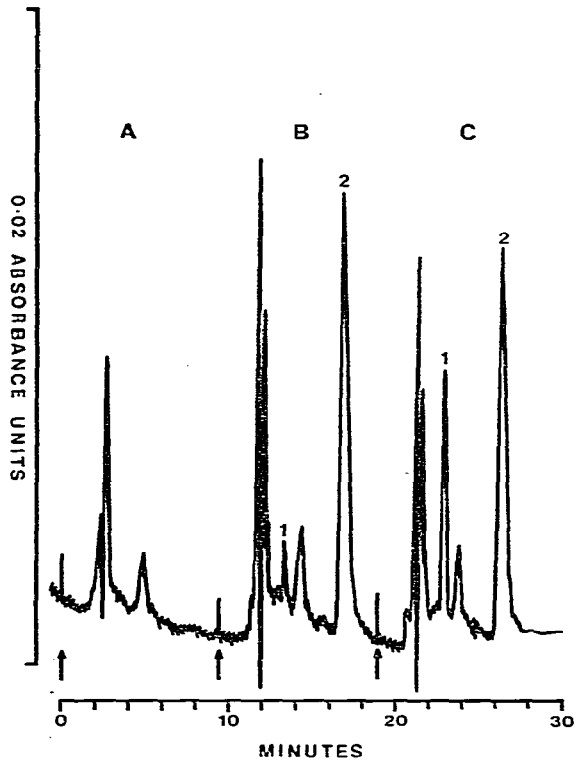

Fig. 1. HPLC traces of a blank plasma extract (A), of a plasma extract spiked with labetalol at a level of 0.08 μg/ml (B) and of a plasma extract spiked with labetalol at a level of 0.32 μg/ml (C). Peaks: 1 = labetalol; 2 = pericyazine (internal standard).

Extraction procedure

To 2 ml of plasma in a 25-ml glass centrifuge tube were added 600 ng of the pericyazine internal standard, 0.2 ml of pH 9.0 buffer and 12.0 ml of diethyl ether. The mixture was shaken for 2 min and centrifuged. A 10.0-ml volume of the organic phase was transferred to a second tube and taken to dryness at 45° under a stream of nitrogen. The residue was dissolved in 100 μl of the elution solvent, and an aliquot injected into the high-performance liquid chromatograph.

RESULTS

Quantitation

To known amounts of labetalol in pointed glass tubes were added 0.6 μg of the internal standard and 100 μl of the eluting solvent. Each was then examined by HPLC using the above conditions. Over the range of 0.09–0.9 μg of the drug the ratio of peak heights of labetalol to the internal standard was linear.

Recovery studies

Amounts of labetalol ranging from 0.09 μg to 0.9 μg were added to 2 ml of blank plasma in order to examine the efficiency of the extraction procedure. The mean recovery of twelve spiked samples (0.045–0.45 μg/ml) was 87 ± 5%.

DISCUSSION

Richards *et al.*[2] have found that the mean peak plasma levels of labetalol from five patients dosed with 200 mg and 400 mg were 0.08 and 0.15 μg/ml, respectively. Because of the large stated dose range, a simple and rapid analytical procedure is needed to monitor the plasma levels.

The method outlined in this paper is specific and no interferences have been found. The sensitivity is such that levels of 0.04 μg/ml can be detected using 2 ml of plasma.

ACKNOWLEDGEMENTS

The authors wish to acknowledge the Commissioner of Public Health, Western Australia, for allowing this work to be published.

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