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Note

Column liquid chromatographic measurement of betaxolol in plasma or serum

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Betaxolol is a cardioseletive β-adrenoceptor blocking drug, with weak membrane-stabilising activity. It has a high oral bioavailability (80%) and a comparatively long terminal elimination half-life (about 16 h) [1,2].

Previous methods for the measurement of betaxolol in biological fluids have used capillary column gas chromatography [3], gas chromatography–mass spectrometry [4,5] and column liquid chromatography (high-performance liquid chromatography, HPLC) with fluorescence detection [6,7]. All of these methods have the required sensitivity to measure the drug following a single intravenous or oral dose. However, they require a relativity large sample volume (0.5–2.0 ml) and involve lengthy extraction procedures which involve either derivatisation prior to analysis [4,5] or evaporation and reconstitution of the organic extract [6,7].

The method described here is based on the direct extraction of 200 μl of sample at alkaline pH into an organic solvent followed by HPLC analysis with fluorimetric detection. It is suitable for the measurement of betaxolol at the concentrations attained following either chronic therapy or single doses.

EXPERIMENTAL

Materials and reagents

Betaxolol [4-(2-cyclopropylmethoxyethyl)-1-phenoxy-3-isopropylamino-2-propanol hydrochloride] was obtained from Lorex Pharmaceuticals (High Wycombe, U.K.). The internal standard, benzimidazole, was obtained from BDH (Poole, U.K.) and was used as a 0.2 mg/l solution in 2 *M* aqueous Tris buffer, pH 9.0, by dilution of a 1.0 g/l methanolic solution of the compound.

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Methanol and methyl *tert.*-butyl ether were HPLC grade (Rathburn, Walkerburn, U.K.). Sodium hydroxide was analytical-reagent grade (BDH). Tris (2 *M*) was prepared in deionised water and adjusted to pH 9 with 1 *M* hydrochloric acid (analytical-reagent grade; BDH; prepared in deionised water). D-10-Camphor sulphonic acid monohydrate was obtained from Aldrich (Gillingham, U.K.).

Column liquid chromatography

A constant-flow reciprocating pump (Applied Chromatography Systems, 750/04) was used with a syringe loading injection valve (Rheodyne 7125, 100-μl loop) and a stainless-steel tube (250×5 mm I.D.) packed with Spherisorb S5W silica (5 μm average particle size) (Hichrom, Woodley, U.K.) at ambient temperature. The column effluent was monitored using fluorescence detection (Kratos-Schoeffel FS 970, excitation 195 nm, no emission filter, time constant 0.5 s) and the integration of peak areas was performed using a Hewlett-Packard 3392A recording integrator. The mobile phase was 1 m*M* camphor sulphonic acid in methanol.

Sample preparation

The sample (200 μl) was pipetted into a small glass (Dreyer) test-tube (50×5 mm I.D.) (Samco, Old Woking, U.K.) and internal standard solution (50 μl) together with methyl *tert.*-butyl ether (200 μl) were added using Hamilton gas-tight syringes fitted with Hamilton repeating mechanisms. The contents of the tube were vortex-mixed for 30 s, following which the tubes were centrifuged (9950 *g*, 2 min; Eppendorf 5412). A portion (approximately 100 μl) of the extract was taken and used to fill the sample loop of the injection valve. Duplicate analyses were performed and the mean result was taken.

Instrument calibration

Standard solutions of betaxolol at concentrations between 25 and 1000 μg/l analyte free-base were prepared in bovine plasma by serial dilution from a methanolic solution of betaxolol hydrochloride equivalent to 1.00 g/l free-base. Internal quality-control samples containing betaxolol at concentrations of 40, 90 and 700 μg/l were prepared in bovine plasma by dilution from an independent stock solution of the drug. These solutions were stable for at least three months when stored at −20°C in the absence of light. On analysis of the standards the ratio of the peak area of betaxolol to the peak area of the internal standard, when plotted against betaxolol concentration, was linear and passed through the origin of the graph.

RESULTS AND DISCUSSION

The chromatography of extracts of a betaxolol plasma standard, drug-free human plasma and of a plasma sample obtained from a patient receiving betaxolol are shown in Fig. 1. No endogenous sources of interference have been observed and analysis of these and other specimens, performed without the addition of benzimidazole, have not revealed the presence of any compounds which

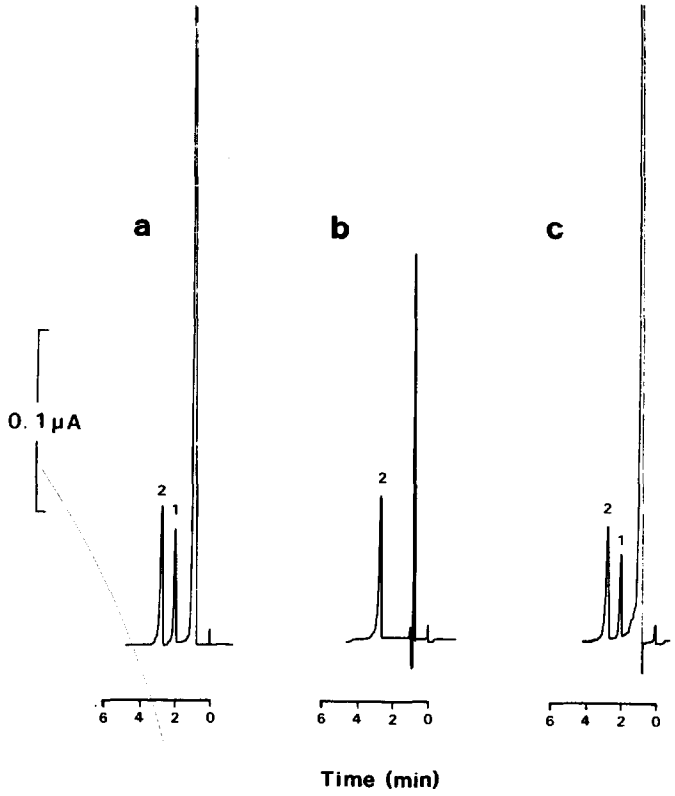

Fig. 1. Chromatograms obtained on analysis of extracts of: (a) a standard solution of betaxolol, at a concentration of 75 μg/l, prepared in bovine plasma; (b) betaxolol-free human plasma; and (c) a plasma specimen from a patient receiving betaxolol (40 mg daily, orally); the betaxolol concentration was 67 μg/l. The initial concentration of benzimidazole was 0.5 mg/l in each case; 100-μl injections. Peaks: 1 = betaxolol; 2 = benzimidazole, the internal standard.

co-elute with the standard. No potential metabolites of the drug have been detected.

Other drugs were studied as potential sources of interference and no compound tested co-eluted with the internal standard, but propranolol co-eluted with betaxolol. The use of an emission filter (370–700 nm) enabled betaxolol to be distinguished from propranolol because it effectively blocked out the fluorescence due to betaxolol. There was no interference from any other drugs tested.

TABLE I

INTRA- AND INTER-ASSAY REPRODUCIBILITY FOR BETAXOLOL ($n = 10$)

	Concentration (μg/l)	Coefficient of variation (%)
Intra-assay	20	5.3
	100	4.1
	250	2.6
Inter-assay	50	4.4

The intra- and inter-assay coefficients of variation (C.V.) for replicate analyses of standard solutions of betaxolol prepared in bovine plasma are shown in Table I. Using a sample size of 200 μl, the limit of accurate measurement for betaxolol in plasma was 5 μg/l (inter-assay C.V. = 9.72%; $n = 10$). The recovery of betaxolol at a concentration of 100 μg/l in plasma was 97.1%, when compared with a methanolic solution of the same concentration ($n = 5$).

This method has been used to measure betaxolol in plasma samples from patients receiving chronic therapy with the drug; concentrations in the range 20–80 μg/l were measured. In addition, the method has sufficient sensitivity to measure the compound following a single oral dose [1].

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