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Note

High-performance liquid chromatographic determination of practolol in plasma

A.C. MEHTA* and R.T. CALVERT

Department of Pharmacy, The General Infirmary, Leeds, Yorkshire (Great Britain)

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Practolol, 4-(2-hydroxy-3-isopropylaminopropoxy)acetanilide, is a cardioselective β-adrenergic blocking agent mainly used for the emergency treatment of cardiac arrhythmias. It is given by a slow intravenous injection of 5 mg, repeated if necessary according to the patient's response. Few methods have been reported for the determination of practolol in biological fluids. They include fluorimetric [1] and gas chromatographic methods [2—4], both requiring derivatization before analysis. Only one high-performance liquid chromatographic (HPLC) method [5] has been published to date utilizing reversed-phase separation and UV detection at 254 nm. This paper describes a new method using a reversed-phase radial compression column with UV detection at 248 nm. Its use in monitoring practolol therapy is also demonstrated.

EXPERIMENTAL

Chemicals and reagents

Liquid chromatography grade methanol, ethyl acetate and analytical reagent-grade ammonia were purchased from BDH (Poole, Great Britain). Practolol and acebutolol hydrochloride were received from ICI (Macclesfield, Great Britain) and May & Baker (Dagenham, Great Britain), respectively.

Apparatus

A reversed-phase column 10 X 0.8 cm, particle size 10 μm, Radial-Pak C-18 (Waters Assoc., Hertford, Great Britain) and a radial compression module, Model RCM-100 (Waters Assoc.), was used in conjunction with a septumless injector, Model U6K (Waters Assoc.), a variable-wavelength UV detector, Model 450 (Waters Assoc.), a pump, Model 110A (Altex Scientific, Berkeley, CA,

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U.S.A.) and a recorder, Model RE 571-20 (Smiths Industries, London, Great Britain).

Procedure

To a stoppered 10-ml tube 1.0 ml plasma sample, 0.2 ml 5 *M* sodium hydroxide, 0.2 ml internal standard (10 μg/ml solution of acebutolol hydrochloride in water) and 5.0 ml ethyl acetate were added. The contents were vortexed for 1 min followed by centrifugation at 750 *g* for 5 min. The ethyl acetate phase was transferred to a tapered tube and evaporated on a sample concentrator at 80°C under a stream of nitrogen. The residue was reconstituted with 200 μl of mobile phase and 80 μl were injected onto the chromatograph.

The following HPLC conditions were used. Mobile phase, methanol—water (75:25) containing 0.03% ammonia (sp. gr. 0.88); flow-rate, 1.8 ml/min; wavelength, 248 nm; detector range, 0.02 a.u.f.s.; injection volume, 80 μl; chart speed, 120 mm/h; temperature, ambient.

Standard solutions of practolol ranging from 0.1 to 1.0 μg/ml were prepared in drug-free plasma and 1.0 ml of each standard was assayed according to the procedure described. The peak height ratio of drug to internal standard was plotted against concentration and the calibration graph was used for measuring the concentration of practolol in the samples.

RESULTS AND DISCUSSION

Chromatograms of spiked plasma as well as a patient's plasma sample of practolol are shown in Fig. 1. The retention times for practolol and acebutolol hydrochloride are 5 and 13 min, respectively. The change in the concentration of ammonia in the mobile phase had a significant effect on the elution of the drug and internal standard (Fig. 2). A concentration of ammonia of 0.03% was found to be optimum under the chromatographic conditions employed. The calibration graph was linear within the above mentioned concentration range. The regression equation for the calibration graph is $y = 0.016 + 1.08x$, $r = 0.999$. Good reproducibility of the method was indicated when ten separate determinations of a 0.25 μg/ml sample of practolol gave a within-day coefficient of variation of 2.4% and a day-to-day coefficient of variation of 5.3%. The lower limit of determination was found to be 30 ng/ml. Percentage recoveries of practolol and acebutolol hydrochloride were calculated by comparing the peak heights of plasma sample (after extraction) with those of aqueous solution containing the same concentration of these compounds. The recovery of practolol at 0.25 μg/ml and acebutolol hydrochloride at 10.0 μg/ml was 75% and 95%, respectively. No peaks were observed at the retention times of the drug or internal standard from the blank plasma sample nor from lignocaine which may be coadministered with practolol. The retention time of lignocaine was 3 min. The presence of 0.03% ammonia in the mobile phase did not cause a reduction of the column efficiency. When stored at 4°C, the plasma standards (calibrators) were stable for four weeks. The method is currently being used to study the pharmacokinetics of practolol in patients with acute myocardial infarction. Fig. 3 shows plasma levels of practolol in a patient following incremental intravenous doses. A total of 20 mg was given in four

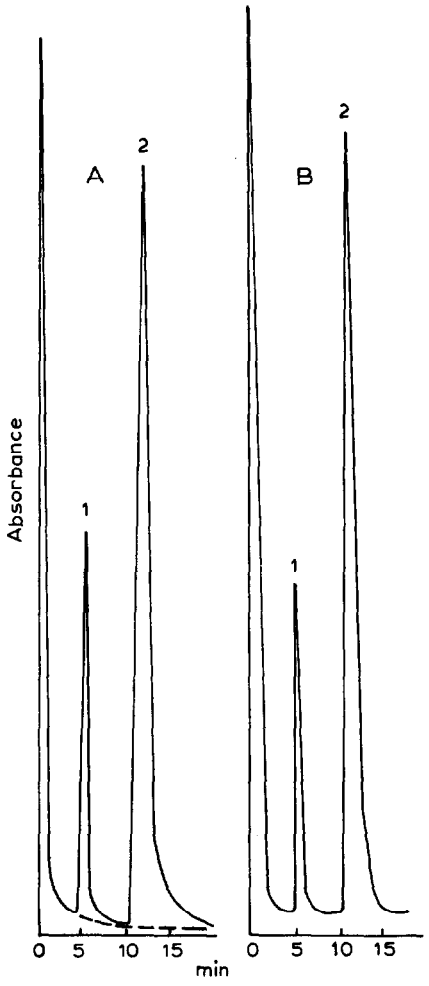

Fig. 1. (A) Chromatogram of plasma standard containing (1) 0.5 μg/ml of practolol and (2) 10 μg/ml of acebutolol hydrochloride. The broken line shows a trace from blank plasma. (B) Chromatogram of a patient's plasma sample containing (1) 0.37 μg/ml of practolol and (2) 10 μg/ml of acebutolol hydrochloride as internal standard.

divided doses every 15 min, the first two doses were 2.5 mg each followed by a 5-mg and a 10-mg dose.

In the previously published HPLC method for practolol [5], a reversed-phase stainless-steel column was used with a mobile phase of ethanol—water (1:9) and a wavelength of 254 nm. No internal standard was used. In the method proposed here the drug is analysed at the wavelength of maximum absorption in presence of internal standard thereby imparting maximum sensitivity and precision to the assay. The mobile phase contains relatively less water and seldom requires deaeration. The radial compression separation system presents a new concept in HPLC. In our opinion radial compression columns afford shorter analysis time, longer life and provide excellent baseline stability. They can be operated at lower pressures and are more economical than the conventional stainless-steel columns.

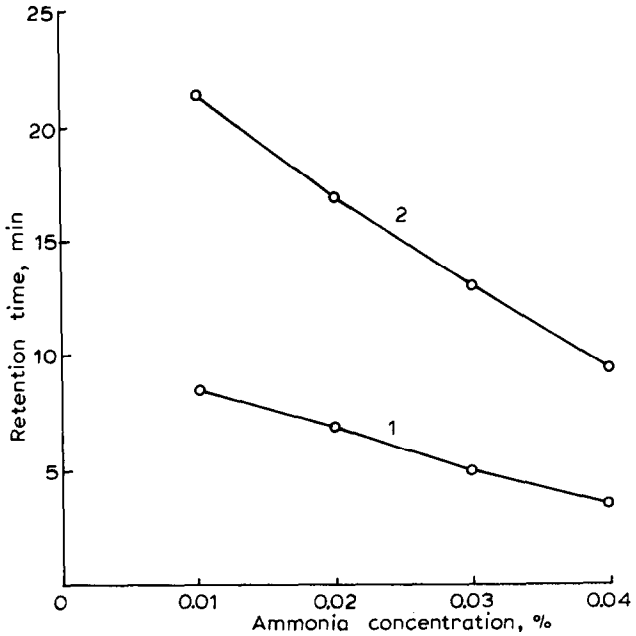

Fig. 2. Effect of ammonia concentration on retention times of (1) practolol and (2) acebutolol hydrochloride.

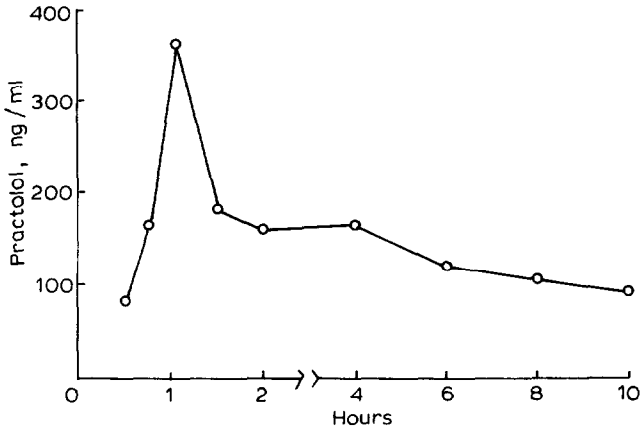

Fig. 3. Plasma profile of practolol from a patient who received repeated intravenous doses totalling 20 mg in 45 min.

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