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Note

Rapid and simple determination of alprenolol in serum

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β-Adrenoceptor blocking drugs (β-blockers) are widely used in the treatment of cardiovascular disorders, such as hypertension, angina pectoris and arrhythmias. Some β-blockers are also successfully used in the treatment of migraine, glaucoma and as minor tranquillizer. Owing to their irregular absorption and extensive first-pass metabolism, serum levels show a great variation, inter- as well as intra-individually. Up to twenty-fold variations in serum levels are reported for propranolol and alprenolol, the latter a more selective β-blocker with intrinsic sympathico-mimetic activity [1—5]. As a result of these variations in serum levels it may be necessary to monitor the drug serum concentration (therapeutic drug monitoring, TDM).

For most of the β-blockers several analytical methods have been described, including gas chromatography (GC) and derivatisation, high-performance liquid chromatography (HPLC) with UV or fluorimetric detection, thin-layer chromatography (TLC) with fluorimetric detection and gas chromatography—mass spectrometry (GC—MS) [6]. An important drawback of most GC methods is the time-consuming derivatisation step necessary to obtain derivatives with a high electron-capture response, introducing a variance due to reaction time and temperature.

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Until now no simple HPLC method that can be used for TDM, pharmaco-kinetic studies or a biopharmaceutical investigation (drug formulation) has been described for alprenolol. Gluth et al. [7] described an HPLC method with UV detection (220 nm) for the determination of oxprenolol, in which they used alprenolol as an internal standard. They gave no information about the detection level and extraction efficiency of alprenolol. HPLC and TLC with UV detection, however, are in general insufficient for the determination of β-blockers in the lower therapeutic range. Johnston et al. [8] described an HPLC method with fluorimetric detection for the determination of metoprolol in plasma, with alprenolol as internal standard. This method may be improved with the use of an excitation wavelength below 230 nm [9].

This paper describes a rapid, simple and sensitive HPLC method, using fluorescence detection at short wavelength, for the determination of alprenolol in serum. The method can be used in TDM or pharmacokinetic studies and is also suitable for the analysis of some other β-blockers.

EXPERIMENTAL

Reagents and chemicals

All chemicals and reagents used were of analytical grade, unless otherwise stated. Water was freshly distilled from deionized tap-water in a glass still.

Alprenolol hydrochloride (Lot No. 196) was kindly donated by Astra Pharmaceutica (The Netherlands) and pyrimethamine (Lot No. 667910) was kindly donated by Hoffmann-La Roche (Switzerland).

Samples

Blood samples taken by venipuncture were clotted for 1 h, then centrifuged at 2300 g for 5 min (Labofuge A, Hereaus Christ, F.R.G.). The serum layer was carefully transferred to a glass tube and stored at −24°C. Contact with plastics was avoided to prevent adsorption of alprenolol or interference with plasticizers [10, 11]. All samples were assayed within two weeks.

Apparatus and conditions

The HPLC system consisted of a Waters Model 6000A constant-flow pump (Etten-Leur, The Netherlands), a Kontron MSI 660 autosampler with a Rheodyne 7010 injection valve equipped with a 100-μl injection loop, and a Schoeffel FS 970 fluorimetric detector (Kratos Analytical, Rotterdam, The Netherlands). A 100 × 3.9 mm I.D. stainless-steel column with Hypersil-ODS (5 μm, Shandon, U.K.) as the stationary phase was used. The eluent was a mixture of acetonitrile (HPLC-grade, Rathburn, Walkerburn, U.K.) and water (25:75, v/v). Triethylamine (TEA; 1 vol.%) was added to the eluent to improve peak symmetry. The apparent pH of the eluent was then adjusted with phosphoric acid to 5.0. The flow-rate was 1.0 ml/min. The excitation wave-length of the fluorimeter was 200 nm, and a 300-nm cut-off filter was placed in the emission beam. The time constant of the detector was set to 4 s, and the amplifier range varied between 0.2 and 0.05 μA full scale deflection, depending on the amount of alprenolol in the samples. Chromatograms were recorded on a 10-mV flat-bed recorder (BD 41, Kipp, Delft, The Netherlands).

A stock solution of alprenolol (10 mg/l) was prepared in methanol and stored at −24°C. From this solution standard solutions were prepared in 0.01 M hydrochloric acid, which were stored between analyses at 4°C. A solution of pyrimethamine (ca. 2 μg/ml) was used as internal standard.

Peak heights were plotted against concentration to give a calibration curve. Correlation coefficients were calculated using linear regression.

Sample preparation

A 0.5-ml volume of a serum sample was pipetted into a 10-ml centrifuge tube. Then 100 μl of the internal standard solution and 0.5 ml of a phosphate buffer (pH 11, 0.5 M) were added to the serum. The samples were vortexed for 30 s with 2.0 ml of n-butyl chloride (glass-distilled grade, Rathburn) and then centrifuged at 2300 g for 2 min. Phase separation was achieved by freezing the water layer and decanting the organic layer into a clean centrifuge tube with a conical bottom. The alprenolol and internal standard were back-extracted with 200 μl of phosphate buffer (pH 1.5, 0.1 M) by vortexing for 30 s. After centrifuging for 2 min (2300 g), ca. 200 μl of the aqueous phase were transferred with a Pasteur pipette to a autosampler vial. n-Butyl chloride traces were removed by briefly centrifuging the autosampler vials, and 100 μl of this solution were injected automatically into the chromatograph.

RESULTS AND DISCUSSION

Although we found 2-propanol to be a satisfactory modifier in the determination of another β-blocker, propranolol [12], we used acetonitrile in this system because it has a lower cut-off wavelength. TEA was further added to the eluent to improve peak symmetry by blocking the silanol groups of the stationary phase [13].

Fig. 1 shows representative chromatograms from extracted blank human serum and a serum sample obtained 60 min after a single oral dose of 100 mg of alprenolol hydrochloride to a volunteer. The concentration in this sample was found to be 18.3 ng/ml alprenolol, calculated as the base. The within-run and between-run variations, with and without internal standard, were checked with spiked serum samples with concentrations of 5 and 50 ng/ml alprenolol hydrochloride. The amounts of alprenolol detected are expressed as a percentage of the spiked amount in Table I. The differences between the calculation with and without internal standard were statistically tested with the Student's t-test for paired results; p values are listed in the table. The low levels of significance indicate that the use of an internal standard is not absolutely necessary.

Pyrimethamine is used as internal standard because alprenolol and pyrimethamine are both completely extracted in the two extraction steps. The log(partition coefficients) (log K_d') for alprenolol and pyrimethamine were 2.7 and 3.1, respectively, in the first basic extraction and −5.1 and −3.01, respectively, in the second acid extraction. Furthermore, pyrimethamine is stable in stock solutions and is readily available in high purity (in contrast to β-blockers).

The use of short excitation wavelengths (190—230 nm) results in a higher sensitivity for compounds containing a phenoxy or an aryloxy group [9]. In
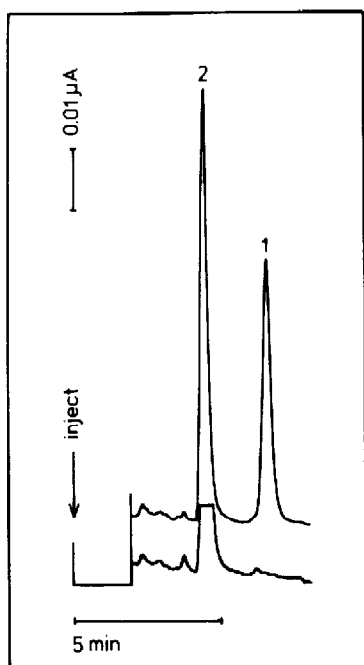
Fig. 1. Chromatograms of blank human serum and of a serum sample taken 1 h after a single dose of 100 mg of alprenolol swallowed by a volunteer with 100 ml of water. Concentration in the sample was 18.3 ng/ml alprenolol, calculated as the base. Peaks: 1 = alprenolol; 2 = internal standard (pyrimethamine).

TABLE I

WITHIN-RUN AND BETWEEN-RUN VARIATIONS OF THE WHOLE ASSAY FOR SERUM SAMPLES SPIKED WITH ALPRENOLOL HYDROCHLORIDE CALCULATED WITH AND WITHOUT INTERNAL STANDARD (I.S., PYRIMETHAMINE)

The results are expressed as percentages of the spiked amounts, mean $\pm$ S.D., $n = 5$.

Concentration (ng/ml)	Variation (%)		P value
	With I.S.	Without I.S.	
Within-run			
5	86.2 ± 1.9	88.6 ± 1.6	0.027
50	102.9 ± 1.5	103.3 ± 1.3	0.680
Between-run			
5	96.3 ± 4.3	101.6 ± 8.2	0.059
50	103.7 ± 4.4	110.2 ± 8.8	0.100

Fig. 2 a plot is presented of the signal-to-noise ratio for alprenolol against the excitation wavelength. On the basis of this result we selected an excitation wavelength of 200 nm. The limit of detection of the whole assay (L_d) was calculated from the baseline noise at maximum amplification of the detector, and defined as twice the peak-to-peak value of the noise. This resulted in a calculated detection limit of 330 pg/ml serum concentration. The recovery of alprenolol and
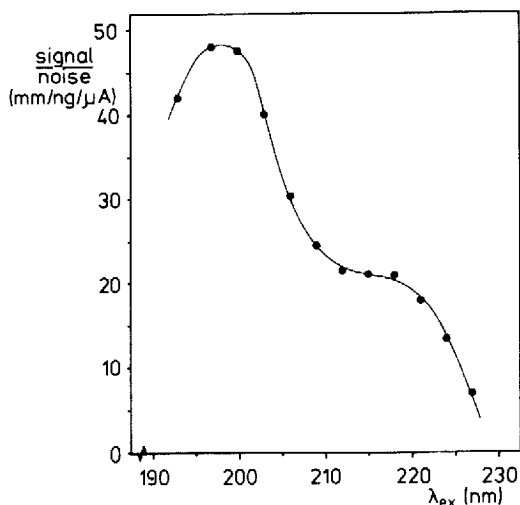
216

Fig. 2. Signal-to-noise ratio of alprenolol peaks at different wavelengths, obtained with multiple injection of the same standard (10 ng). Excitation wavelength, 193—227 nm; emission wavelength, > 300 nm.

the linearity of the whole system were checked with spiked human blank serum. The detector response is linear in the range from 0 to at least 400 ng/ml alprenolol serum concentration. The recovery of alprenolol was calculated from calibration curves, made on separate days, by comparing the slope of the calibration curve with the slope of a curve obtained after direct injection of the aqueous standards. The mean recovery (± S.D.) for alprenolol was 89.3 ± 2.0% (n = 6). No corrections were made for the loss of alprenolol by the solubility of water in n-butyl chloride.

No interference of the following drugs, which may be used in combination with β-blockers, could be found: furosemide, hydralazine, chlorothiazide, procainamide, quinidine, disopyramide, lignocaine, tocainide, acetyl salicylic acid, acetaminophen, caffeine, diazepam and chlordiazepoxide. Quinidine, lignocaine, procainamide and diazepam had all a capacity factor smaller than 2, furosemide (capacity factor ca. 8) was not extracted and the other drugs did not fluoresce in this system or had an infinitely high capacity factor.

The analytical method described is also suitable for the determination of

TABLE II

CAPACITY FACTORS OF SOME OTHER COMMON β-BLOCKERS IN THE CHROMATOGRAPHIC SYSTEM DESCRIBED

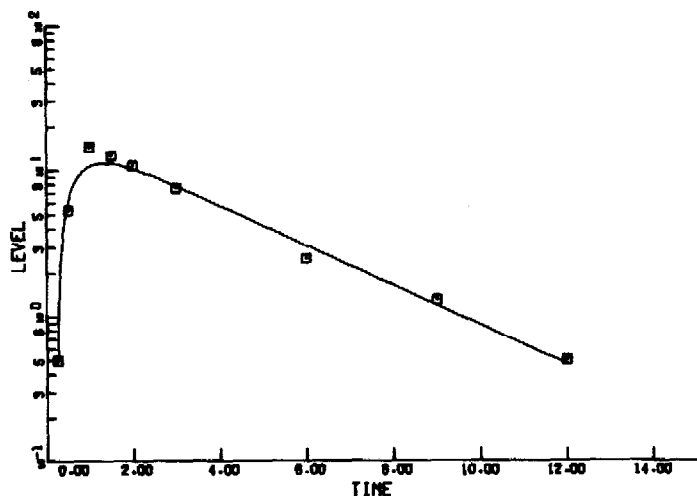| β-Blocker	Capacity factor
Acebutolol	≈1
Atenolol	<1
Alprenolol	5.31
Metoprolol	1.13
Oxprenolol	2.32
Propranolol	4.92
Sotalol	<1
Timolol	No response

Fig. 3. Serum concentration—time curve for alprenolol after a single oral dose of 100 mg of alprenolol hydrochloride.

other β-blockers. Only minor modifications in the eluent composition and extraction are necessary. Table II lists the capacity factors of some other common β-blockers in this system.

Fig. 3 shows a concentration—time curve obtained after a single oral dose of 100 mg of alprenolol hydrochloride swallowed by a volunteer with 100 ml of water after an overnight fast. The following pharmacokinetic parameters were calculated from the data with FARMFIT, assuming a single compartment: elimination rate constant (k_{el}), 0.314 h^{-1}, elimination half-life ($t_{1/2}$), 2.20 h; maximum concentration (C_{max}), 11.2 ng/ml; time to reach C_{max}, 1.34 h. Serum levels after a single dose of alprenolol are relatively low owing to the extensive first-pass metabolism of the drug.

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