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Note

# Simple and rapid determination of propranolol and its active metabolite, 4-hydroxypropranolol, in human plasma by liquid chromatography with fluorescence detection

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In recent years different methods for the determination of propranolol [1—3], and metabolites [4—6] in plasma using liquid chromatography (LC) with fluorescence detection have been reported. These methods attain a very high sensitivity (concentration lower than 1 ng/ml can be quantified) but they usually require an additional evaporation step [1—3, 5, 6] and/or back-extraction to concentrate and purify the extracts [2, 4, 6]. The monitoring of plasma levels of propranolol and 4-hydroxypropranolol is useful in some clinical situations to adjust the therapy and to study the correlations between doses and clinical effects that still have to be clarified completely, especially in some neurological pathologies [7, 8].

Since in long-term oral therapy low doses (120 mg/day) of propranolol are able to produce plasma levels of the parent drug and of 4-hydroxypropranolol greater than 10 ng/ml [9, 10], we developed a method sensitive enough for the therapeutic monitoring of these compounds in chronically treated patients which is simpler and faster than the previously published methods.

## MATERIALS AND METHODS

### *Reagents and standards*

Propranolol and 4-hydroxypropranolol were kindly supplied by Imperial Chemical Industries (Macclesfield, Great Britain). Desimipramine was kindly supplied by Geigy (Basle, Switzerland). Methanol and water were for LC use (Baker, Deventer, The Netherlands). Other reagents and solvents were of analytical grade and were checked for chromatographic purity before use.

A 0.03 *M* solution of acetate buffer was prepared as follows: 1.8 ml of

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glacial acetic acid were added to 1000 ml of water, the pH was adjusted to 5.5 ± 0.2 with 6 *N* sodium hydroxide and the solution was filtered through a 0.22-μm membrane filter (GS type, Millipore, Malsheim, France) for subsequent use in the preparation of the LC mobile phase.

*Standard solutions*

Stock solutions of propranolol, 4-hydroxypropranolol and desimipramine were prepared in methanol to give a concentration of 1 mg/ml for each compound. Working solutions of propranolol and 4-hydroxypropranolol (1 μg/ml) were made by diluting the relative stock solutions with methanol; a working solution of desimipramine (500 ng/ml) was made by diluting the relative stock solution with acetonitrile. All solutions were stored at −20°C.

Plasma standards of 10, 25, 50, 100 and 250 ng of propranolol and 4-hydroxypropranolol per ml of plasma (calibration samples) were prepared at the time of analysis as follows: exact volumes of working solutions of both compounds in test-tubes were evaporated to dryness under a gentle stream of nitrogen; to these samples, 0.9 ml of drug-free plasma and 0.1 ml of 0.1 *M* sodium metabisulphite in water were added.

*Biological materials*

All materials used throughout the analysis were made of glass or steel, to avoid interaction between biological matrix and plasticizers [11].

Blood (about 7 ml) from patients was collected into heparinized glass tubes and the plasma was immediately separated; 2 ml of plasma were transferred to another test-tube and 0.2 ml of 0.1 *M* sodium metabisulphite in water was added to avoid degradation of 4-hydroxypropranolol [2]. Samples were stored at −20°C until analysed. The final results were corrected for dilution.

*Apparatus and conditions*

A Series 3 liquid chromatograph, an LC-100 column oven, a 204-A fluorescence spectrophotometer (all from Perkin-Elmer, Norwalk, CT, U.S.A.), a Hibar LiChrosorb CN (10 μm) column, 25 cm long (Merck, Darmstadt, G.F.R.) and an HP 3380-A recording integrator (Hewlett-Packard, Avondale, PA, U.S.A.) were used.

The mobile phase was prepared by mixing 65 parts of methanol with 35 parts of 0.03 *M* acetate buffer. This solution was degassed before use in an ultrasonic bath (Branson, Soest, The Netherlands). The mobile phase flow-rate was 1.5 ml/min, the column temperature was 55°C. The spectrophotometer was set at 285 nm (excitation) and 405 (emission); the slit openings both for excitation and for emission were 20 nm.

*Extraction procedure*

To 1 ml of biological material (patients' plasma or calibration samples) were added 1 ml of the marker solution (500 ng/ml desimipramine in acetonitrile) and about 0.2 g of a mixture (4:1) of sodium chloride and sodium carbonate. The samples were vortexed for 20 sec then centrifuged at 1500 *g* for 5 min at 4°C. The clean upper layer, which was primarily acetonitrile, was injected directly into the chromatographic system.

## RESULTS AND DISCUSSION

The method described is similar to the LC method for the determination of acidic or neutral drugs recently published [12], based on protein precipitation by acetonitrile—salt pair, centrifugation and direct injection of the upper liquid phase into the chromatographic system.

Typical chromatograms obtained from a blank plasma and that of a patient are shown in Fig. 1. Calibration curves (from 10 to 250 ng/ml) showed a linear correlation between concentration and peak area ratios: $y = 0.020\ x + 0.006$, $r = 0.999$ for propranolol; and $y = 0.005\ x + 0.013$, $r = 0.995$ for 4-hydroxypropranolol. To calculate these data a least-squares linear regression method was used. The mean coefficient of variation for the analysis of six calibration curves (30 samples), performed over a 2-month period, was 5.5% for propranolol and 6.3% for 4-hydroxypropranolol. Absolute recoveries were 97% for propranolol and 109% for 4-hydroxypropranolol. By injection 30 $\mu$l of the extracts, concentrations of 10 ng/ml of each compound can be quantified. If samples at concentrations lower than 10 ng/ml have to be analyzed, they can be injected twice at two different settings of the fluorescence detector: excitation wavelength 285 nm for both substances, emission wave-

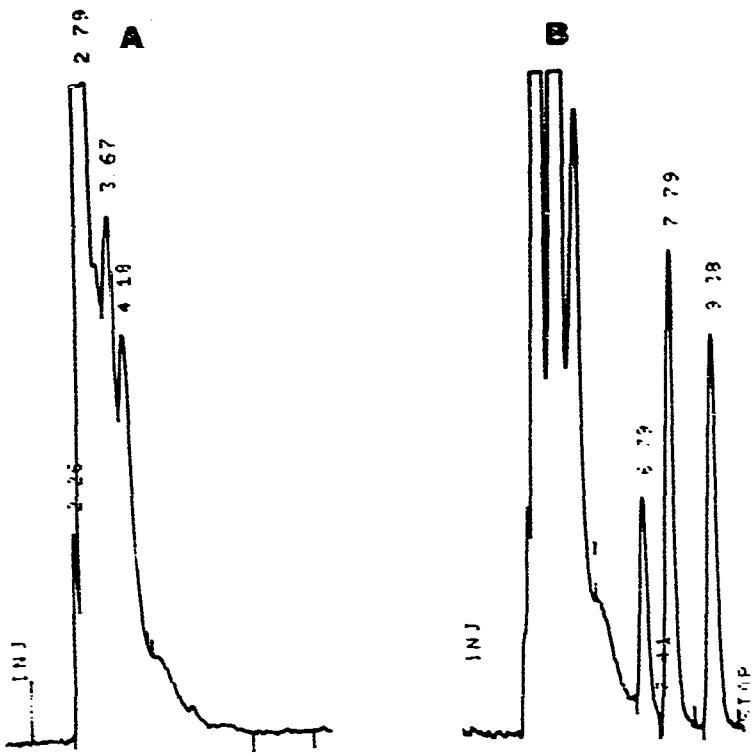


Fig. 1. Liquid chromatographic responses obtained by injecting 15 $\mu$l of (A) extract from a blank plasma, (B) extract from a patient's plasma. The peaks are (retention time): 6.79 min = 4-hydroxypropranolol (31.2 ng/ml); 7.79 min = propranolol (66.5 ng/ml); 9.38 min = desimipramine (marker).

length 345 nm for propranolol and 420 nm for 4-hydroxypropranolol. Under these conditions the minimum detectable concentration of both compounds is lower than 5 ng/ml.

We checked, without observing any chromatographic interferences, some drugs (procainamide, quinidine, metoprolol, phenytoin, carbamazepine, atenolol, methysergide and dihydroergotamine) so that the plasma of patients assuming these drugs in combination may be analyzed. Moreover, over a period of some months of routine monitoring of the plasma levels of propranolol and 4-hydroxypropranolol in patients treated with propranolol for the therapy of essential tremor and migraine, no drugs, metabolites or endogenous plasma constituents were found to interfere with the analysis. The present procedure omits the evaporation and purification steps and is thus faster and simpler than the previous methods for routine therapeutic monitoring of propranolol and 4-hydroxypropranolol.

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