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Note

# High-performance liquid chromatographic method for the determination of labetalol in plasma using ultraviolet detection

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Labetalol, 2-hydroxy-5-[1-hydroxy-2-(methyl-3-phenyl-propylamine)ethyl]-benzamine hydrochloride, is a competitive alpha- and beta-adrenergic receptor antagonist [1—4], which has been shown to be effective in the treatment of hypertension [5—8]. The blockade of the alpha-adrenergic receptors in the periphery produces a fall in both systolic and diastolic blood pressure, but a reflex increase in heart rate and cardiac output (baroreceptor-mediated) is prevented by the stronger blocking of the beta receptors. A linear correlation between the plasma concentration and the pharmacological effects of labetalol has been reported [9]. In common with many beta blockers, labetalol is extensively metabolized in the liver, with less than 5% of the dose being excreted unchanged in the urine [10]. Reduced bioavailability of orally administered labetalol due to first-pass metabolism has been indicated [11]. Inter-subject variability in the extent of the first-pass elimination is pronounced and accounts for the large differences in the area under the curve observed between individuals upon administration of the same oral dose of labetalol [11].

Several assays for the measurement of labetalol in biological fluids have been published, the usefulness of which varies with the intended application. The present method involves reversed-phase high-performance liquid chromatography (HPLC) and UV detection of labetalol and the internal standard, propranolol. It possesses the sensitivity and specificity necessary for undertaking the studies that would allow further elucidation of the pharmacokinetic characteristics of this drug. The method is also suitable for monitoring labetalol levels in a routine clinical situation.

## MATERIALS AND METHODS

### *Reagents*

Labetalol hydrochloride was supplied by Schering (Bloomfield, NJ, U.S.A.) and propranolol hydrochloride was supplied by Aldrich (Milwaukee, WI, U.S.A.). Methanol and diethyl ether (anhydrous), both HPLC grade, were obtained from MCB Manufacturing Chemist (Cincinnati, OH, U.S.A.) and J.T. Baker (Phillipsburg, NJ, U.S.A.), respectively. The ammonium acetate and ammonium phosphate were reagent grade.

### *Instrumentation*

An Altex Model 110A solvent metering pump (Altex, Berkeley, CA, U.S.A.), was used as the high-pressure solvent delivery system. The samples were injected with an Altex Model 210 sample injection valve, and their absorbance was measured with an Altex Model 155/10 variable-wavelength UV detector. Separation was achieved on an Ultrasphere octyl 5-$\mu$m (150 × 4.6 mm) analytical column (Altex). The absorbances of labetalol and the internal standard were measured at 216 nm (maximum for labetalol).

### *Chromatographic conditions*

The mobile phase consisted of 0.01 *M* ammonium phosphate—methanol (1:1) and the pH of the mixture was adjusted to 3.0 with 15 *M* phosphoric acid. A flow-rate of 1 ml/min was used, generating a back-pressure of approximately 1800 p.s.i. (125 bar). The column and mobile phase were maintained at room temperature.

### *Procedure*

To a 15-ml disposable glass tube containing 0.75 ml of plasma, serum or whole blood, 100 $\mu$l of internal standard solution (propranolol, 850 ng/ml) and 0.25 ml of 1 *M* ammonium acetate buffer (pH 9.0) were added. The samples were extracted with 6 ml of diethyl ether by vortexing for 1 min, and separation of the organic layer was achieved by centrifugation at 1500 *g* for 5 min. The organic layer was transferred to a 15-ml screw-capped conical centrifuge tube containing 100 $\mu$l of 0.1 *M* hydrochloric acid solution. Labetalol and the internal standard were back-extracted into the aqueous phase by vortexing for 1 min, and the layers were again separated by centrifugation at 1500 *g* for 5 min. A 50-$\mu$l aliquot of the aqueous phase was injected onto the column.

## RESULTS

Under the specified conditions the retention times of labetalol and the internal standard were 5 and 9 min, respectively. The chromatogram of 0.75 ml of plasma containing 200 ng/ml labetalol and 85 ng/ml propranolol is shown in Fig. 1A. Fig. 1B shows the chromatogram from the plasma of a patient receiving long-term labetalol therapy for hypertension. After analyzing 0.75 ml of plasma of a person receiving no medication, no interference was seen at all (Fig. 2). The limit of detection using 0.75 ml of plasma is 10 ng/ml. The precision and accuracy of the assay are shown in Table I. As it can be seen,

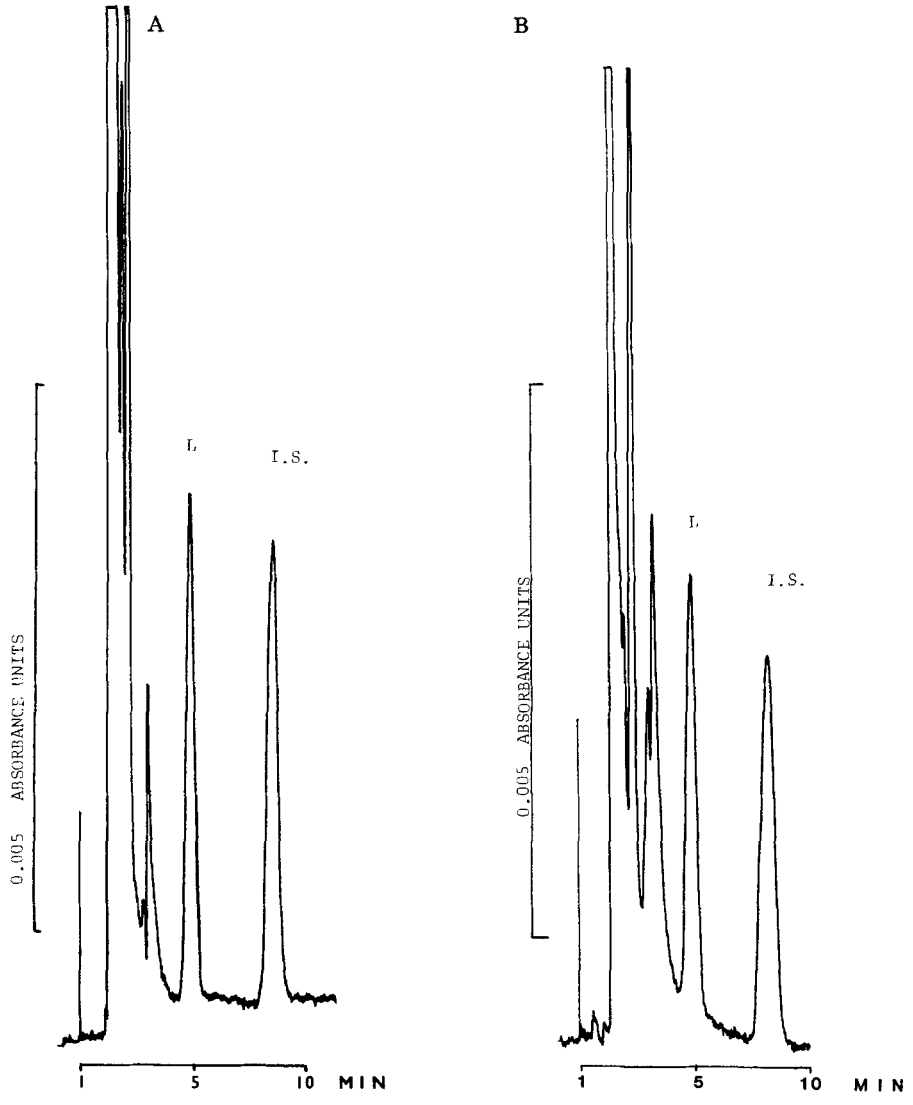


Fig. 1. Chromatograms of (A) 0.75 ml of plasma containing 200 ng/ml labetalol (L) and 85 ng/ml propranolol, internal standard (I.S.) processed as described in the text; (B) plasma sample from a hypertensive patient who was receiving labetalol.

intraday and interday assay coefficients of variation (C.V.) were 4.4% and 7.8%, respectively, at 50 ng/ml and 4.5% and 5.5% at 200 ng/ml. No deviations from linearity were seen over the concentration range 10—1000 ng/ml. The intraday recovery of a 100 ng/ml labetalol serum solution was 80.5% (C.V. = 4.5%, $n$ = 10) and the interday recovery of the same solution was 81.4% (C.V. = 4.8%, $n$ = 7). The selectivity of the assay was assessed by assaying several drugs which may present considerable likelihood of being co-administered with labetalol. As can be seen in Table II most drugs did not show any peak. In the few cases in which a peak was present, the differences in retention time avoided

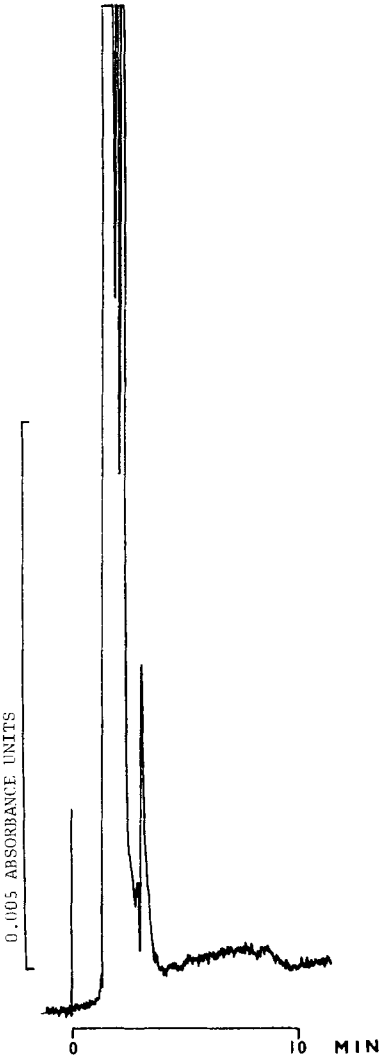


Fig. 2. Chromatogram of 0.75 ml plasma blank processed as described in the text.

possible interferences. Under these experimental conditions, the analytical column has lasted for over 5000 injections.

## DISCUSSION

Several analytical methods have been proposed for the determination of labetalol and/or its metabolites in serum [12—16]. Most of these methods, however, contain certain inherent limitations which might limit their general application. Since the plasma concentration of labetalol appears to be related to the pharmacological activity of this drug, the accurate quantitation of the blood levels is useful in optimizing clinical response. Many previous studies on the pharmackinetics of labetalol [9, 12, 17—19] used a spectrophotofluorimetric assay [12] which may have limitations since it does not involve prior

TABLE I

INTRA- AND INTERDAY REPRODUCIBILITY OF REPLICATE STANDARDS AT TWO CONCENTRATIONS

| | Within-day | | Between-day | |
|---|---|---|---|---|
| | 50 ng/ml | 200 ng/ml | 50 ng/ml | 200 ng/ml |
| | 49.1 | 208.8 | 53.5 | 224.0 |
| | 53.1 | 212.7 | 55.2 | 193.9 |
| | 53.1 | 215.0 | 44.8 | 207.5 |
| | 55.1 | 198.8 | 47.5 | 223.6 |
| | 53.1 | 223.5 | 49.2 | 208.4 |
| | 49.1 | 199.5 | 48.5 | 219.0 |
| | 51.1 | 209.7 | | |
| $X$ = | 52.0 | 209.7 | 49.8 | 212.6 |
| S.D. = | 2.3 | 9.5 | 3.9 | 11.6 |
| C.V. = | 4.4 | 4.5 | 7.8 | 5.5 |

TABLE II

THE RETENTION TIME OF SOME DRUGS LIKELY TO BE CO-ADMINISTERED WITH LABETALOL THAT WERE ANALYZED BY USING THE ASSAY DESCRIBED

| Drug | Retention time (min) | Drug | Retention time (min) |
|---|---|---|---|
| Flurazepam | 6 | Chlorpromazine | No peak |
| Doxepin | No peak | Digoxin | No peak |
| Dilantin | No peak | Nortriptyline | No peak |
| Librium | 6.3 | Desipramine | No peak |
| Phenobarbital | No peak | Chlorthalidone | No peak |
| Imipramine | No peak | Amitriptyline | No peak |
| Quinidine | 4 | Trifluprom azine | No peak |
| Furosemide | No peak | Procainamide | No peak |
| Diazepam | No peak | Lidocaine | No peak |

chromatographic separation. Three HPLC assays have been published [13—15] in which use is made of spectrophotofluorimetric detectors. Their sensitivity is high and good selectivity is also obtained. Nevertheless, all of these methods utilize more tedious methodology; e.g., multiple extractions, dry-ice freezing, chromic acid washing, nitrogen-drying of large volumes (15 ml) of diethyl ether [14] and instrumentation; e.g., post-column alkalinization [15], which are not required by the procedure described herein. In addition fluorescence detectors are somewhat less available than variable-wavelength UV detectors. This might be a limitation if reproduction or utilization of these fluorescence assays is intended. Only one analytical procedure has been reported [16] which combines HPLC separation with UV detection. The sensitivity limit of this assay limits it to the routine clinical monitoring of relatively high serum concentrations and the lack of a back-extraction makes it more susceptible to interferences. It was not reported if the assay had been used to analyze real patient samples.

The assay presented herein, involves extraction of alkalinized samples into an organic solvent (diethyl ether) and then back-extraction into an acidic solution. The extraction of potentially interfering acidic compounds is therefore minimized in the first step and the back-extraction of neutral compounds will be minimized in the second step. The preparation of the samples is fast and their rapid chromatographic analysis allows for the analysis of over 25 samples in a regular work-day. The assay allows for the determination of labetalol in plasma as well as in whole blood. This is particularly important since we have determined from in vitro studies using human blood with 25% hematocrit that labetalol accumulates approximately three-fold in human erythrocytes relative to the plasma concentration. This accumulation will undoubtedly be more significant in vivo when the hematocrit approaches 50%.

The rapidity of the described assay together with its sensitivity and selectivity should make it a strong candidate for routine clinical drug analysis when a rapid determination of labetalol plasma levels is desired. This assay was used to measure labetalol plasma concentrations of ten hypertensive patients who had been receiving chronic labetalol therapy. There were no interferences observed and the plasma levels ranged from 30 to 800 ng/ml. At the same time, the accuracy and sensitivity allows the precise quantitation of blood levels required for detailed pharmacokinetic studies to be undertaken. This method is currently being utilized to investigate first-pass interactions of labetalol with other high-extraction ratio drugs.

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