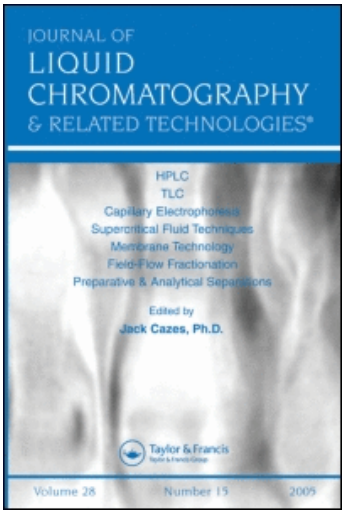

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Labetalol Analysis in Human Plasma Using Liquid Chromatography with Electrochemical Detection: Application to Pharmacokinetic Studies

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LABETALOL ANALYSIS IN HUMAN PLASMA USING LIQUID CHROMATOGRAPHY WITH ELECTROCHEMICAL DETECTION: APPLICATION TO PHARMACOKINETIC STUDIES

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ABSTRACT

Labetalol determination in human plasma by a sensitive (to 2.5 ng/ml) and selective method using liquid chromatography with electrochemical detection is described. Plasma is extracted with diethyl ether under mildly basic (pH 9) conditions, back-extracted into an aqueous acidic buffer, then injected directly on column. Standard curves using propranolol as an internal standard are linear for concentrations from 2.5 to 200 ng/ml. Within-day and between-day reproducibility is satisfactory with coefficient of variation less than 8% for all concentrations. Sample recovery from the extraction is complete at all concentrations. Utility of the method is demonstrated by a pharmacokinetic study in a hypertensive volunteer who received 43.75 mg labetalol by 10 minute intravenous infusion.

INTRODUCTION

Labetalol, an adrenergic antagonist which exhibits beta-1, beta-2, and alpha-1 blocking properties (1), has recently been

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introduced into clinical use for the treatment of hypertension (2). Methods for its analysis in biological samples have included a radioreceptor assay (3), spectrofluorimetry (4), high pressure liquid chromatography using fluorimetric detection (5-9), and high pressure liquid chromatography using UV detection (10,11). These methods have been limited in sensitivity to about 10 ng/ml labetalol concentration in plasma due to limited detector sensitivity. This is satisfactory for many applications, however when evaluating labetalol single-dose pharmacokinetics, it is useful to accurately and precisely detect lower concentrations to more rigorously define the slope of the terminal elimination phase.

Martin et al. (12) observed that labetalol can undergo oxidation and is therefore amenable to electrochemical detection after liquid chromatographic separation. This detection technique is inherently more sensitive than the above-listed methods, with sensitivity of analysis for some endogenous substances as low as 25-50 pg/ml (13). Here we report the sensitive and selective analysis of labetalol in human plasma using liquid chromatography for separation and electrochemical oxidation as a means of detection.

METHODS

Apparatus and Chromatographic Conditions

The high-pressure liquid chromatographic system consisted of a Waters (Waters Assoc., Milford, MA, USA) model A-6000 solvent delivery system with a Waters Assoc. model U6K sample loop. The separation system was a 3.9 mm x 30 cm stainless steel (10 micron) C-18 micro Bondapack reversed phase column (Waters). The detector was an ESA model 5100 A (Environmental Sciences Assoc., Inc., Bedford, MA, USA) dual electrode coulometric electrochemical detector. The guard cell was ESA model 5020 and the detector cell ESA model 5010. Guard cell voltage was +1.05 V, detector 1 of the dual electrode analytical cell +0.40 V, and detector 2, +1.00 V.

Signal from detector 2 was used as output, which was quantitated on a Fisher (Fair Lawn, NJ, USA) Model 5000 chart recorder.

Mobile phase (68% distilled water, 32% acetonitrile, with 10 ml glacial acetic acid added per liter) was run at a flow rate of 1.5 ml/min. All analyses were performed at room temperature.

Reagents

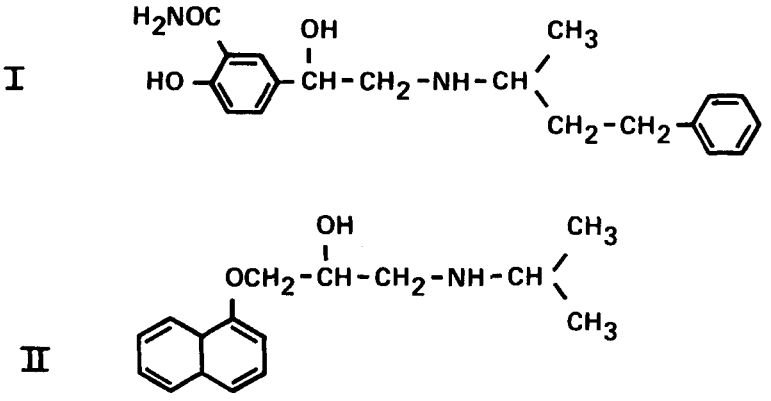
Pure labetalol was kindly provided by Glaxo, Inc. (Research Triangle, NC, USA) and the internal standard propranolol by Ayerst Co. (New York, NY, USA) (Figure 1). All other reagents analytical grade or better, were purchased from commercial sources without further purification. Water was deionized, double-distilled, and then prepared mobile phase filtered through a 0.2 micron filter (Rainin Instrument Co. Woburn, MA, USA) and degased under vacuum.

Reference Standards

Standard solutions were prepared by dissolving the appropriate quantity of labetalol hydrochloride to yield 100 mg labetalol base in 100 ml methanol. The internal standard propranolol was prepared in a similar manner. Sequential dilutions to 1 µg/ml were then made. These solutions were stored at 4°C and are stable for at least six months.

Preparation of Samples

Propranolol was used as the internal standard. A 100 µl volume of the stock solution (1 µg/ml), containing 100 ng propranolol was added to a series of 14 ml (110 x 17 mm) conical polyethylene centrifuge tubes with tight sealing polyethylene caps (Sarstedt Scientific, FRG). A 0.2 to 2.0 ml sample of unknown plasma was added to each tube. Labetalol calibration standards



I. Labetalol
II. Propranolol

FIGURE 1. Structural formulae of labetalol (I) and the internal standard propranolol (II).

were prepared by adding 5, 10, 25, 50, 100, and 200 ng of drug to consecutive tubes containing "blank" plasma. One "blank" sample, taken from the patient being studied prior to drug administration, is analyzed with calibration standards and each set of unknown samples.

Extraction Procedure

One milliliter of ammonium acetate (1 M) buffer pH 9 was added to each plasma sample prior to extraction to permit all extractions at a mildly basic constant pH. Five milliliters diethyl ether was then added to each tube. The tubes were agitated gently by hand for five minutes, then centrifuged at 4°C for five minutes at 400 g. The organic layer was transferred to another 14 ml polyethylene centrifuge tube which contained 100 µl 1% phosphoric acid. This mixture was again agitated gently by hand for five

minutes and centrifuged at 4°C for five minutes at 400 g. The upper organic layer was discarded. Tubes were then left open in room air for 30 minutes to assure evaporation of all diethyl ether from the aqueous phase. Ten to 50 µl of aqueous phase was then injected directly into the sample loop.

Clinical Pharmacokinetic Study

A hypertensive 31 year old male patient participated in a pharmacokinetic study of intravenous labetalol after giving written informed consent. The patient received a single dose of approximately 50 mg labetalol hydrochloride (Glaxo, Research Triangle, NC, USA) equivalent to 43.75 mg (quantitated by the method reported here) base by infusion into an antecubital vein over 10 minutes. Multiple venous blood samples were drawn into "Venoject" heparin-containing tubes (Terumo Medical Corp., Elkton, MD, USA) over the following 24 hours. Plasma was separated by centrifugation (400 g x 10 minutes; 4°C) and concentrations of labetalol were determined by the method described above.

Plasma labetalol concentrations were analyzed by iterative weighed nonlinear least-squares regression analysis (14,15). After correction of the derived coefficients for the time of infusion (16), the following pharmacokinetic variables for labetalol were determined: distribution half-life, elimination half-life, mean residence time, central compartment volume, total volume of distribution at steady state and by the area method, and total clearance.

RESULTS

Evaluation of the Method

Under the described chromatographic conditions, labetalol and propranolol gave symmetric well resolved chromatographic peaks

(Figure 2). Electrochemical detector oxidation voltage for maximum sensitivity for both labetalol and propranolol was optimized (Figure 3) by sequential injection of known amounts of standards at different analytical cell potential settings. The first electrode of the analytical cell was used to oxidize background contamination prior to oxidation of drug or internal standard which was then monitored on electrode 2. Therefore electrode 1 oxidation potential was set at +0.40V, below the potential required for any oxidation of drug or internal standard (Figure 3).

Drug-free blank plasma samples were consistently free of endogenous contaminants at the retention times corresponding to labetalol and propranolol. The relation of plasma labetalol concentration to the labetalol:propranolol peak height ratio was linear to 200 ng/ml.

Analysis of 17 standard curves over an eight-month period indicated the correlation coefficient of peak height ratio to labetalol concentration was always .993 or greater. Day-to-day coefficient of variation in the slope of the calibration curve was 9.2%.

The limit of sensitivity of the method is 2.5 ng/ml of a 2 ml extracted plasma sample. Within-day coefficients of variation for identical samples of labetalol were at 5 ng/ml, 6.6% (n=5); 10 ng/ml, 8.0% (n=6); 25 ng/ml, 5.2% (n=5); 50 ng/ml, 3.7% (n=6); 100 ng/ml, 3.9% (n=6); and 200 ng/ml, 4.0% (n=6). Residue analysis indicated the extraction of labetalol was 98 ± 4% (n=3) at 25 ng/ml and 101 ± 3% (n=3) at 100 ng/ml extracted concentrations when labetalol was added in these concentrations to 1 ml plasma prior to extraction.

Pharmacokinetic Study

Figure 4 shows plasma labetalol concentrations and the pharmacokinetic function for the described subject. Derived pharmacokinetic variables are listed in Table 1.

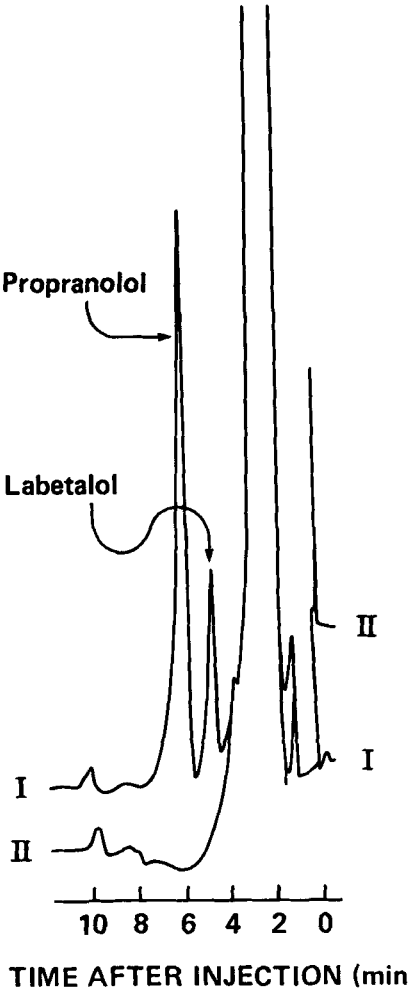


FIGURE 2. Liquid chromatogram of the extract of 1 ml plasma obtained from the patient prior to receiving labetalol (I) and an extract of 1 ml plasma obtained after drug administration (II).

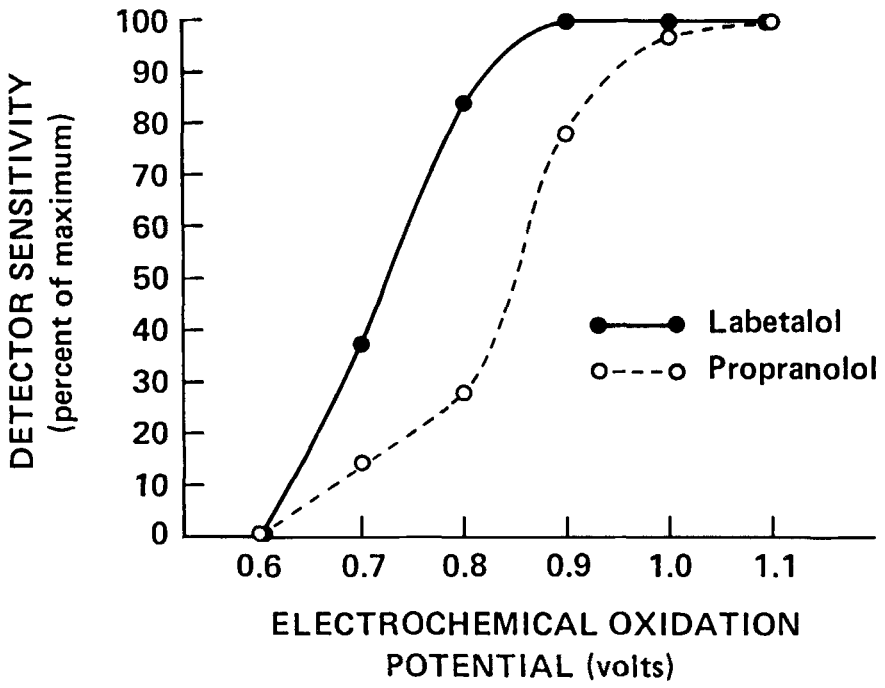


FIGURE 3. Extent of electrochemical oxidation of increasing oxidation protentials of the analytical cell for labetalol (●—●) and propranolol (O---O).

TABLE 1

Derived Labetalol Pharmacokinetic Variables After a Single 50 mg Intravenous Dose to a Hypertensive 31 Year Old Male

Distribution Half-Life (minutes)	6.8
Elimination Half-Life (hours)	2.62
Mean Residence Time (hours)	1.86
Central Compartment Volume (liters/kg)	1.5
Volume of Distribution at Steady State (liters/kg)	2.34
Volume of Distribution by Area (liters/kg)	4.75
Total Clearance (ml/min/kg)	21.0

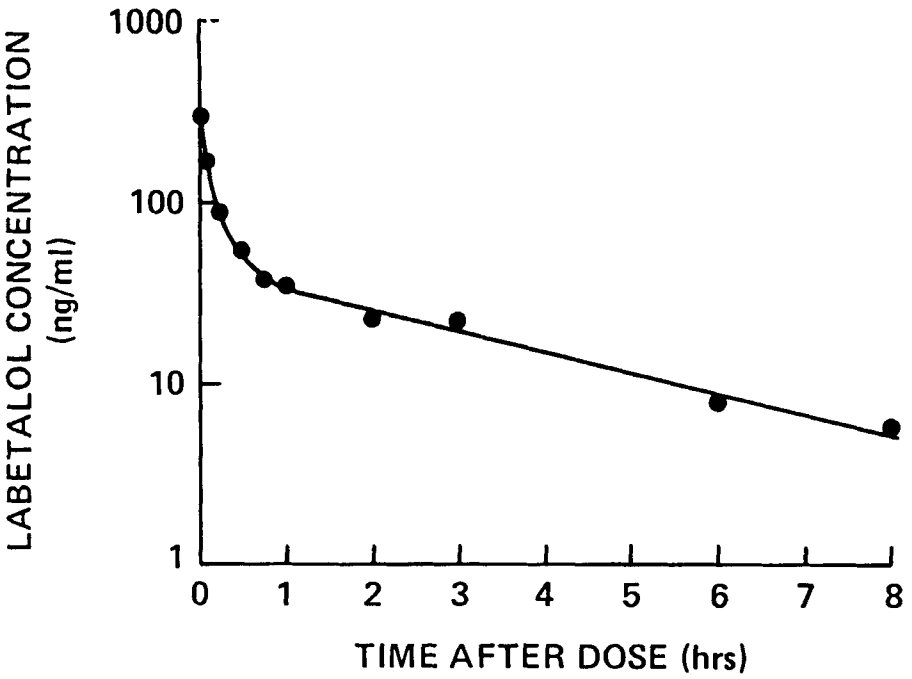


FIGURE 4. Plasma labetalol concentrations and the derived labetalol pharmacokinetic function following intravenous labetalol hydrochloride administration to a hypertensive but otherwise healthy 31 year old male patient. See Table 1 for derived pharmacokinetic variables.

DISCUSSION

A reliable, sensitive, and selective method is described for quantitation of labetalol in human plasma by high-pressure liquid chromatography using electrochemical detection. Mildly basic extraction conditions are used (here pH 9) to optimize recovery due to the limited lipophilicity of labetalol and its amphoteric character (17). Blank human plasma samples are free of contaminants in the areas corresponding to the retention times for labetalol and propranolol. Metabolites identified to date in the human are very polar glucuronides which do not accumulate and are

rapidly excreted in the urine (18), therefore do not interfere with this method in plasma.

Advantages of this method over previously described analyses are its sensitivity and the minimal sample preparation required. During a normal working day about 40 samples can be extracted and chromatographed. In addition use of the electochemical detection system and the ease with which these two beta-adrenergic antagonists are detected using it suggests that electrochemical oxidation may be a technique with wider application for analytical determinations in this important drug class. This is of considerable interest due to the inherent high degree of sensitivity attained (13) when coulometric electrochemical oxidation is employed.

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