Journal of Chromatography, 344 (1985) 412—415
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 2720

Note

Measurement of labetalol by high-performance liquid chromatography with electrochemical detection

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(First received March 15th, 1985; revised manuscript received May 17th, 1985)

Labetalol, 2-hydroxy-5-[1-hydroxy-2-(1-methyl-3-phenylpropylamino)ethyl] benzamide hydrochloride, is a combined α- and β-adrenergic receptor antagonist, which has been shown to be clinically effective in the treatment of hypertension [1, 2]. For pharmaceutical studies and routine clinical monitoring of the labetalol, a sensitive and reliable method for its determination is desirable. Similar to other β-blockers, labetalol is extensively metabolized in the urine, with less than 5% of the dose being excreted unchanged in the urine [3]. Different liquid chromatographic methods, using ultraviolet [3, 4] or fluorimetric [5, 6] detection, have been described for measurements of labetalol in biological fluids. Electrochemical detection of labetalol following liquid chromatography has not been attempted, despite its inherent sensitivity and the redox behavior of the drug. The development of such a detection scheme for labetalol, its characteristics and applications are described in this paper.

EXPERIMENTAL

Apparatus

The liquid chromatographic system (Bioanalytical Systems LC-303) consisted of a dual-piston pump (PM-30A), a Rheodyne Model 7125 injector (20-μl loop), a Biophase ODS 5-μm reversed-phase column (25 cm × 4.6 mm), and an amperometric detector (Model LC-3) equipped with a thin-layer glassy carbon transducer. The reference electrode was an Ag/AgCl (Model RE-1, Bioanalytical Systems). The system was operated at ambient temperature. Cyclic voltammetry was performed in a 10-ml voltammetric cell using a Princeton Applied Research Model 264A voltammetric analyzer. The flow injection system was described earlier [7], except that a 20-μl sample loop was used.

0378-4347/85/$03.30

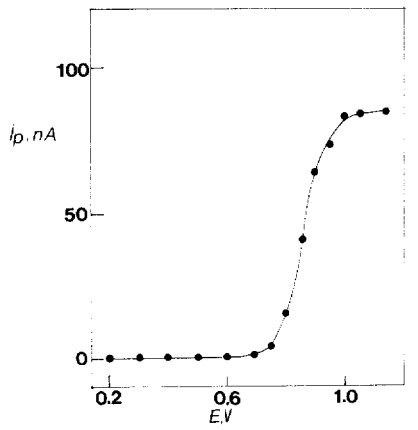
Reagents

All aqueous solutions were prepared in double-distilled water. Stock solutions of labetalol hydrochloride (Sigma) were made up fresh each day. The mobile phase was acetonitrile–0.05 *M* dipotassium hydrogen phosphate (35:65), adjusted to pH 6 with phosphoric acid. The mobile phase contained 0.2 g/l Na_2EDTA. A 0.05 *M* phosphate buffer (pH 7.4), prepared from a 1:4 mixture of potassium dihydrogen phosphate and dipotassium hydrogen phosphate served as supporting electrolyte and carrier solution in the flow-injection experiments. The urine samples were obtained from a healthy volunteer, filtered by passing through a glassy filter (10–15 μm porosity), degassed and diluted with the mobile phase solution.

RESULTS AND DISCUSSION

To determine the optimum potential for the assay of labetalol, a hydrodynamic voltammogram was generated by repetitive 200-ng injections of the drug (Fig. 1). A well defined wave, attributed to the oxidation of the hydroxy moiety, is observed. Maximum (mass transport limited) signal is obtained at potentials higher than +1.0 V. However, the selection of the best operating potential requires a trade-off between the signal and the problems of background current, noise and baseline drift. Accordingly, lower potentials (+0.9 to +0.95 V) were preferred and used throughout this study.

The pH of the mobile phase affects the labetalol peak current and retention time (Fig. 2). Changes of the pH over the 3.5–7.0 range result in a gradual increase in retention time from 6.1 to 8.5 min, and a maximum peak current near pH 6; this pH was used throughout. The increase in peak current over the



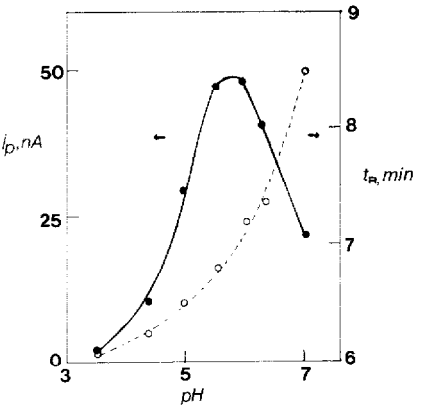


Fig. 1. Hydrodynamic voltammogram for injections of 200 ng labetalol. Flow-rate, 1.0 ml/min; mobile phase, acetonitrile–0.05 *M* dipotassium hydrogen phosphate (35:65) adjusted to pH 6 with phosphoric acid.

Fig. 2. Dependences of the peak current (•) and retention time (○) on the pH of the mobile phase. Injections of 200 ng labetalol; operating potential, +0.9 V; flow-rate and mobile phase as in Fig. 1.

3.5—6.0 pH range is attributed in part to changes in the redox behavior. Cyclic voltammograms for 20 mg/l labetalol performed in mobile phase solutions of pH 6.0 and 4.5 yielded an anodic peak at potentials of +0.83 V and +0.93 V, respectively. Thus, more positive operating potentials would be required to attain the mass-transport limited signal in solutions of decreasing pH. The voltammograms recorded over the +0.1 to +1.4 V range revealed only an oxidation peak, indicating an irreversible redox process (not shown; scan rate 100 mV/sec). Such batch voltammetric measurements may be used when simple samples (e.g., pharmaceutical tablets) are concerned. For example, a well defined differential pulse peak was obtained for $1 \cdot 10^{-6} M$ labetalol at a carbon paste disk electrode (not shown; scan-rate, 5 mV/sec; amplitude, 50 mV).

The sensitivity and linearity of the electrochemical detection for liquid chromatography were evaluated using six successive injections of labetalol solutions of increasing amount (5—30 ng, not shown). The peak response increased linearly with increasing amounts of the drug; the slope of the resulting calibration plot corresponded to a sensitivity of 0.19 nA/ng (correlation coefficient, 0.999; intercept, −0.07 nA). The detectability is illustrated in Fig. 3, that shows a chromatogram for an injection of 2 ng labetalol. Based on a signal-to-noise ratio of 3, these data correspond to a detection limit of 0.6 ng (20-μl injection). These data indicate the suitability of the method for trace measurements of labetalol. The precision of the results was estimated by six repeated injections of a 30-ng labetalol solution (operating potential, +0.95 V; other conditions as in Fig. 1). The mean peak response found was 5.7 nA, with a range of 5.4—6.1 nA, and a relative standard deviation of 5%. The detectability and precision were evaluated also in electrochemical detection for a flow-injection system, that offers the speed advantage desirable for quality control tests in the pharmaceutical industry. Detection limits of 0.1 ng (signal-to-noise ratio of 3) and a relative standard deviation of 1.1% (n = 10) were estimated from injections of 1.8- and 20-ng labetalol solutions, respectively. The lower detection limit obtained in these measurements is attributed to the use of gravity flow that results in lower noise level.

The suitability of the present method for direct measurements in biological fluids is demonstrated in Fig. 4. A urine sample, spiked with labetalol at the

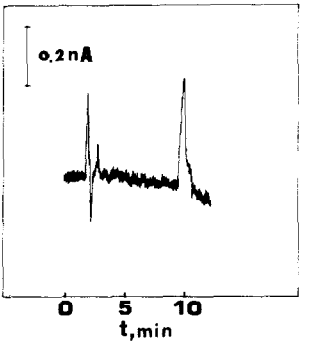


Fig. 3. Chromatogram for an injection of 2 ng labetalol. Operating potential, +0.95 V; other conditions as in Fig. 1.

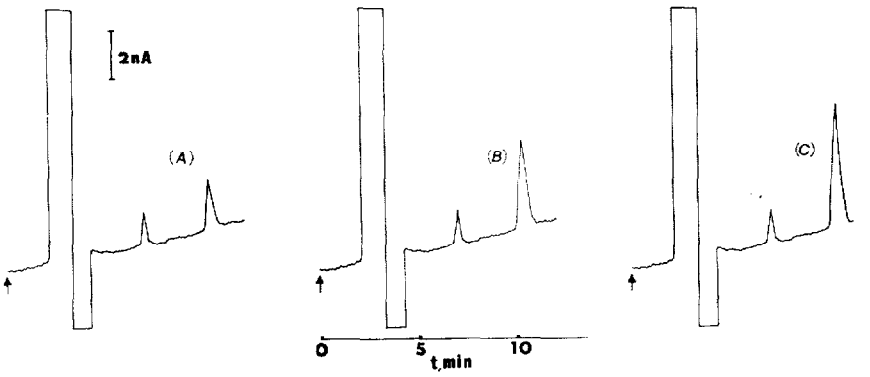


Fig. 4. Chromatograms obtained after spiking a urine sample with labetalol at (A) 6.25 μg/ml; (B) 12.5 μg/ml and (C) 18.75 μg/ml levels, and diluted 1:25 with the mobile phase. Conditions as in Fig. 2.

6.25—18.75 μg/ml level and diluted 1:25 (5—15 ng injected), was used without any preliminary treatment, except for filtration. (The use of a precolumn is recommended under these conditions to prolong the life of the analytical column.) The labetalol peak (t_R = 10 min) is not affected by other electroactive constituents of the urine sample that are eluted earlier. The absence of interferences obtained in untreated urine samples is attributed to the selectivity of the electrochemical detection. Direct proportionality between the peak current and the labetalol level is observed. Similar response was obtained using samples collected from different volunteers.

ACKNOWLEDGEMENTS

This work was supported by the American Heart Association and the National Institutes of Health (Grant No. GM 30913-01A1).

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