Liquid-Chromatographic Analysis for Esmolol and Its Major Metabolite in Urine

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We describe a simple, reproducible liquid-chromatographic method for determination of esmolol (a short-acting beta blocker) and its major metabolite in human urine. Esmolol is extracted from urine at a pH of 8.4 into methylene chloride; the more polar metabolite remains in the aqueous phase. We then measure esmolol with a \( \mu \)Bondapak C18 column and measure ultraviolet absorbance at 229 nm; the metabolite is analyzed with a Spherisorb phenyl column, with absorbance measured at 280 nm. The average extraction recoveries of esmolol and the metabolite were 95 and 92%, respectively. Standard curves were linear and reproducible for esmolol from 0.025 to 5 mg/L and for the metabolite from 1 to 250 mg/L. Within-day CVs for both compounds were <5%.

Esmolol (Breviblock™; Figure 1, I), a short-acting beta adrenergic blocking agent, is rapidly metabolized by esterases in the blood (I). In humans it is eliminated with an average half-life of 9 min (2).

Methods reported for the analysis for esmolol include gas chromatography–mass spectrometry, for blood (3), and "high-performance" liquid chromatography (HPLC), for pharmaceutical preparation (4, 5). Recently an HPLC method has been described for determination of the metabolite of esmolol in blood (6). To date, however, no analysis for esmolol (I) and its major metabolite (II, Figure 1) in urine has been reported. To assess the extent of metabolism and the excretion of unchanged drug, a suitable assay method is needed. Here we describe a simple, reproducible HPLC method for quantifying I and II in human urine. We also report the amounts of I and II measured in the urine after administration of esmolol hydrochloride to healthy volunteers to evaluate the utility of this assay procedure.

Materials and Methods

Chemicals

Compounds I and II and the internal standards, ACC-9038 (methyl 4-[4-(2-hydroxy-3-(2-methylethyl)amino)propoxy]phenyl]butyrate hydrochloride) and ACC-8059 [1-(3-aminopropionic acid)-3-(4-chlorophenoxy)-2-propanol hydrochloride], were synthesized at American Critical Care, McGaw Park, IL. "HPLC-grade" acetonitrile and methylene chloride were purchased from J. T. Baker Chemical Co., Phillipsburg, NJ. Glacial acetic acid, phosphoric acid, monobasic potassium phosphate, sodium acetate, mono and dibasic sodium phosphate, and sodium borate were of A grade and were purchased from commercial sources.

Reagents

Standard solutions. We prepared solutions of I and II in distilled water, in concentrations covering the range of the respective standard curves and the internal standard solutions ACC-9038 (20 mg/L) and ACC-8059 (400 mg/L).

Phosphate buffer (pH 6.2): per liter, 10 mmol of monobasic sodium phosphate and 5 mmol of dibasic sodium phosphate. Adjust to pH 6.2 with phosphoric acid.

Borate–phosphate buffer (pH 8.4). Mix 38 mL of 0.2 mol/L monobasic potassium phosphate solution with 62 mL of 0.1 mol/L sodium borate solution.

Mobile phase A. A 6535 (by vol) mixture of phosphate buffer (pH 6.2) and acetonitrile, final pH 6.9.

Mobile phase B. A 91/8/1 (by vol) mixture of 10 mmol/L sodium acetate, acetonitrile, and glacial acetic acid, final pH 3.4.

Apparatus

We used an HPLC system (all from Waters Associates, Milford, MA) consisting of a Model 6000A pump, a Model 440 ultraviolet detector with an extended-wavelength module, and a Model 710 B WISP automatic sample injector. Column A was Waters \( \mu \)Bondapak C18, 300 mm × 3.9 mm (i.d.). Column B was Spherisorb phenyl, 10-\( \mu \)m particle size, 250 mm × 4 mm (i.d.) from Alltech Associates, Deerfield, IL. Chromatograms were recorded with a strip-chart recorder (Linear Instruments Corp., Reno, NV). Peak-height ratios and all concentrations were calculated by the HP-3356 laboratory automation system (Hewlett-Packard, Palo Alto, CA).

Procedures

Extraction of urine. Transfer 1 mL of urine into a 20 mm × 125 mm glass culture tube (with a Teflon-lined screw cap) containing 10.0 mL of methylene chloride, 1.0 mL of the borate–phosphate buffer, 0.1 mL (2 \( \mu \)g) of ACC-9038 (internal standard for I) and 0.15 mL of water. Tightly cap the tube, vortex-mix for 10–15 s, and shake mechanically for 10 min. Centrifuge (1900 × g, 10 min), then transfer the organic-solvent phase into a 16 mm × 125 mm glass culture tube and add 1.0 mL of a 0.25 mol/L solution of monosodium phosphate (pH 2.8). Shake the tube mechanically for 10 min, centrifuge for 10 min, and transfer the clear aqueous layer into a "WISP" vial for injection into the HPLC for analysis for I. Meanwhile, transfer 0.5 mL of the aqueous phase from the initial extraction into a 13 mm × 100 mm glass culture tube, add 0.1 mL (40 \( \mu \)g) of ACC-8059 (internal standard for II) and 0.5 mL of dilute acetic acid (150 mL of glacial acetic acid per liter), and mix. Add 5.0 mL of methylene chloride,

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\begin{align*}
\text{CH}_3\text{CHNHCH}_2\text{CHOCH}_2\text{O} & \quad \text{(CH)}_2\text{COOR} \\
\text{I: } \text{R} = \text{CH}_3 \\
\text{II: } \text{R} = \text{H}
\end{align*}
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Fig. 1. Chemical structures of esmolol (I) and its metabolite (II)

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then shake mechanically for 10 min, centrifuge for 10 min to clarify the layers, and transfer the aqueous phase into a WISP vial for HPLC analysis for II.

Elute compound I from column A with mobile phase A and measure the absorbance at 229 nm. Elute II from column B with mobile phase B and measure the absorbance at 280 nm. Use a flow rate of 2.0 mL/min for both mobile phases. Calculate the peak height ratios of I and II to the respective internal standards and determine their concentrations by reference to the respective standard curves. The concentrations of I and II are expressed in terms of their hydrochloride salts.

Preparation of standard curves. We prepared standard curves for each subject on the same day the samples were analyzed. Standard curves were prepared by use of both undiluted and diluted urine as necessary. Before the extraction step we added, instead of 0.15 mL of water, 0.1 mL of I and 0.05 mL of II (in various concentrations) to the 1.0 mL of blank urine to yield final respective analyte concentrations in the range of 0.025 to 5 mg/L and 1 to 250 mg/L.

Analytical recovery and precision studies. We added known amounts of I and II to blank urine samples and compared the respective peak heights of I and II in the extracted urine samples with those obtained from direct injection of standard solutions containing equivalent amounts of I and II.

Within-day assay precision was assessed by sextuplicate determinations of low, medium, and high concentrations of I and II. Day-to-day precision was determined on four to six different days.

Clinical Samples

Urine specimens were from healthy, male volunteers who were taking part in esmolol clinical studies. The specimens were collected before, during, and after administration of the drug and analyzed according to the method described here.

Results and Discussion

The extraction procedure described here provided excellent and reproducible analytical recoveries of both I and II. I, a weak base, is unstable in aqueous solution at alkaline pH. The analytical recovery of I was maximum and independent of pH in the pH range of 7.3–8.6. At pHs greater than 8.6, the recovery decreased somewhat, presumably because of decomposition of I. To optimize the extraction of I from urine samples of widely differing pHs (4.7–7.8), we stabilized the samples by adding borate-phosphate buffer (pH 8.4).

Mean analytical recovery of I from urine containing 0.1 to 4 mg of the compound per liter was 96% (range 92–99%). Mean analytical recovery of II from urine containing 10 to 100 mg/L was 92% (range 87–97%).

Figures 2 and 3 show representative chromatograms obtained for extracts of urine samples before and after administration of I to a human volunteer. Internal standards were added to the after-drug urine samples. Under the HPLC conditions we used, the retention times for I, ACC-9038, II, and ACC-8059 were 6.1, 8.2, 10.8, and 6.0 min, respectively. The chromatographic peaks were sharp, and I and II were well resolved from their respective internal standards. Extracts of blank urine did not show any extraneous peaks eluting in the regions where compounds I, II, or ACC-9038 were eluted. A small interfering peak was eluted at the same time as ACC-8059, but we minimized the contribution of this interferent to the peak height of ACC-8059 by increasing the concentration of ACC-8059 added to the urine. Furthermore, the interfering peak was not seen when diluted urine samples were used, as was the case for most clinical samples.

The present assay method provided reproducible results.
for both I and II. Precision was assessed at concentrations of 0.025, 0.5, and 5 mg/L for I and 1, 10, and 250 mg/L for II. CVs for within-day and day-to-day assay of I ranged from 2.9 to 4.2% and 9.3 to 14.9%, respectively. The corresponding data for II were 3.7 to 6.1% and 6.1 to 20.0%.

For the day-to-day precision studies, we used urine samples from different volunteers. Some samples showed a small peak eluting in the region of II, thus contributing to the slightly greater variability, at the lower concentrations, in the day-to-day analysis for II. However, because urine samples for the assay of II required a 50- to 100-fold dilution to bring the concentration within the range of the standard curve, the interference was effectively eliminated. For diluted urine, the CV for day-to-day assay of II ranged from 1 to 8%.

Standard curves prepared on different days were linear and reproducible over the concentration range of 0.025–5 mg/L for I and 1–250 mg/L for II. For both standard curves, coefficients of determination (r²) were 0.998 or greater.

To demonstrate the usefulness of this assay procedure, we collected and assayed urine samples from volunteers receiving a 6-h infusion of esmolol hydrochloride at 300 µg/kg body weight per minute. We confirmed that I is extensively metabolized. On average, <1% of the unchanged dose was accounted for in urine collected 0 to 36 h after the beginning of the infusion, whereas the metabolite, II, accounted for about 77% of the administered dose. Figure 4 illustrates the cumulative recoveries of I and II from urine from a typical volunteer.

The present assay is simple, reproducible, and sensitive enough for use in evaluating metabolism and urinary recovery of I in humans. We have analyzed more than 3000 urine samples obtained during several different clinical studies and found the method suitable for quantifying I and II. In our experience, column A has performed satisfactorily for over 500 samples, column B for 1000 samples. The inlet frit, however, has had to be cleaned or replaced after every 200–250 samples.

References