Determination of Propranolol in Plasma by Radial Compression Liquid Chromatography with Fluorometric Detection

Adnan El-Yazgi and Cazemiro R. Martin

This radial compression liquid-chromatographic assay for propranolol in plasma is rapid, reproducible, and suitable for use in routine monitoring. A 10-μm particle, 8 mm × 10 cm CN cartridge is used in conjunction with a radial compression separation system. The mobile phase is monobasic sodium phosphate (pH 3) solution/methanol/acetonitrile (760/84/156 by vol), the flow rate 6 ml/min. Propranolol was detected by use of a spectrofluorometer equipped with a 20-μL flow-through cell, at excitation and emission wavelengths of 250 and 336 nm. The retention times for propranolol and metoprolol (the internal standard) are 3.13 and 1.42 min, respectively. A one-step extraction with chloroform yields "clean" chromatograms, with >99% of the drug being analytically accounted for. Under these conditions, results are precise and accurate. Currently we are using this method to monitor propranolol in hypertensive neonates. Data on changes in the concentrations of propranolol in plasma with time are presented for one such patient.

Reagents

Monobasic sodium phosphate, 85% phosphoric acid, methanol, aconitrite, chloroform, and hexane (all from Fisher Scientific Co., Fair Lawn, NJ) were all of "HPLC" grade. Before use, we first extracted, with chloroform, a saturated solution of propranolol HCl (ICI Ltd., Macclesfield, Cheshire, U.K.) in 3 mol/L sodium hydroxide, and recrystallized the drug several times (m.p. 77.3 °C) from hexane. Metoprolol tartrate (Ciba Labs., Horsham, Switzerland) was pharmaceutical grade. The water used was a "reverse osmosis" water passed through a trace-organic removal cartridge and 0.45-μm (av pore size) membrane filter (Millipore Co., Milford, MA 01757).

The mobile phase was prepared by mixing 760 mL of 50 mmol/L monobasic sodium phosphate (adjusted to pH 3 by dropwise addition of 85% phosphoric acid) with 156 mL of aconitrite and 84 mL of methanol. The solution was filtered through a 0.45-μm membrane and degassed before use. The mobile phase was prepared every day or so, and was stable over this period. Thus, no change in the retention time was noticed.

Procedures

Apparatus conditions. The mobile phase was pumped at a flow rate of 6 mL/min and a pressure of 7.9 MPa (1150 psig). The chart speed of the recorder was set at 0.5 cm/min. Excitation and emission wavelengths of the spectrophotofluorometer were 250 and 336 nm, respectively, and the spectral bandwidth was 10 nm. Under these detection conditions, sensitivity was maximum. The signal gain was set at 10 (maximum is 100).

Extraction of drug from plasma. To 1 mL (or less) of heparinized human plasma, add 60 μL of internal standard solution (metoprolol tartrate, 100 mg/mL) and 100 μL of 2 mol/L NaOH. Next, add 9 mL of chloroform, vortex-mix for 30 s, and centrifuge for 8 min at 2800 rpm. Aspirate and discard the upper (aqueous) layer, and transfer 8 mL of the chloroform layer to a clean conical tube for evaporation under a gentle stream of nitrogen. Redissolve the residue in 150 μL of the mobile phase, placing the tube in an ultrasonic water bath for 2 min, then transfer the sample to the micro-vial of the auto-sampler, which is programmed to inject 50 to 75 μL into the cartridge.

Standard curve. To 1-mL portions of human plasma, add appropriate aliquots of either 0.04 or 5 mg/L solutions of propranolol in 20 mmol/L HCl to yield final propranolol concentrations of 1, 20, 100, 250, 375, and 500 μg/L. Add the internal standard and NaOH as described above, then extract and chromatograph the samples. Construct the standard curves by plotting the peak height ratio (propranolol/internal standard) vs the concentration of propranolol.

Accuracy and precision. We analyzed in quadruplicate blank plasma samples supplemented with 20, 100, 250, 375, and 500 μg of propranolol per liter, and computed the analytical recovery as 100 × amount found/amount added.
We calculated the within-assay CV from quadruplicate analyses of plasma samples containing 25, 100, and 400 μg of propranolol per liter.

**Extraction efficiency.** We determined the efficiency of the chloroform extraction procedure by comparing the peak heights for propranolol in plasma samples supplemented with various amounts of propranolol with those for equal amounts of propranolol in stock HCl solution.

**Patient's Samples**

Heparinized plasma samples were collected at different time intervals from a 3.05-kg female neonate who was being treated for hypertension in the Neonatology Clinic. She was receiving 0.15-mg doses of propranolol, administered by rapid intravenous injection. We assayed her samples as described above and calculated the concentrations of propranolol by comparison with the standard curve.

**Results and Discussion**

Figure 1 depicts representative chromatograms of blank plasma, propranolol-supplemented plasma, and patient's plasma samples. Propranolol and metoprolol tartrate (the internal standard) were eluted rapidly, with retention times of 3.13 and 1.4 min, respectively. The peaks of both compounds are sharp and symmetrical, indicating a high chromatographic efficiency.

The standard curves were highly reproducible and linear. The correlation coefficients for curves constructed on eight different days ranged from 0.9986 to 0.9992. The use of chloroform to extract propranolol from plasma yielded clean chromatograms with an extraction efficiency >90% at various concentrations.

We determined the accuracy of the assay at various concentrations of propranolol as described above. Analytical recovery ranged from 100.8% to 104.3%.

For low, normal, and above-normal concentrations of propranolol in plasma, the means were 27.8 (SD 0.4 μg/L, CV 1.4%), 108 (SD 0.5 μg/L, CV 0.5%), and 402 (SD 3.9 μg/L, CV 1%), respectively.

We examined the interference in the assay by determining the retention times for 15 drugs chromatographed under the conditions described. The solutions were prepared by dissolving arbitrary amounts of these drugs in about 1 mL of mobile phase. None of the following, which were detected fluorometrically, appeared to interfere: digitoxin, theophylline, coumarin, procainamide, acetylsalicylic acid, nicotine, phenytoin, furosemide, epinephrine, nadolol, thiamine HCl, labetalol.

Table 1 lists propranolol concentrations measured at various intervals after the intravenous administration of 0.15 mg of propranolol to a neonate. There was an initial rapid decrease in concentration, followed by a slow decline. To our surprise, we observed a secondary "rebound" peak, suggesting an enterohepatic recycling with lag-time (4) for this drug in this patient. This has not been previously reported for propranolol in neonates, and merits further investigation. A comprehensive study of the pharmacokinetics of propranolol in hypertensive patients is in progress in our laboratory, but will not be reported for some time, given the rarity of this condition in neonates.

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**References**


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<th>Time after dose, h</th>
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