Liquid-Chromatographic Determination of Nadolol in Plasma

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We describe a liquid-chromatographic procedure for determining nadolol in plasma. After an analog of nadolol is added as internal standard, the plasma sample is passed through a disposable BondElut C18 column. After several column washes, nadolol and the internal standard are eluted with methanol, and the eluate is evaporated and reconstituted with the mobile phase (acetonitrile/water, perchloric acid, and tetramethylammonium hydroxide). An aliquot of the extract is chromatographed on a non-silica resin-base reversed-phase column. The peaks are detected by fluorescence ($\lambda_{ex} = 265$ nm and $\lambda_{em} = 305$). Drug and internal standard are well resolved, and only a few extraneous peaks appear. The standard curve ranges from 10 to 400 µg/L. We are using this procedure to determine steady-state concentrations of nadolol in patients receiving various dosages of nadolol along with other types of antihypertensive drugs.

Additional Keyphrases: chromatography, reversed-phase • fluorometry • antihypertensive drugs • “beta blockers”

Nadolol (Figure 1), a new, nonselective beta adrenergic receptor blocker, has been recently approved in North America for treatment of hypertension and angina pectoris. Unlike other “beta blockers,” it is not metabolized to any great extent in the liver, being excreted largely unchanged in the urine, and it has an unusually long biological half-life, 20–24 h (1). Because nadolol is less able to cross the blood–brain barrier, it is therefore less likely to cause adverse effects such as lassitude and mental depression.

Most of the pharmacokinetic data for this drug have been obtained by a fluorometric procedure (2) in which the drug is oxidized with periodate and the resulting dialdehyde is condensed with o-phenylenediamine to produce a fluorescent product. This procedure has been validated by a gas-chromatographic/mass-spectrometric (GC-MS) procedure (3). We have tried to develop a simple chromatographic procedure for the routine monitoring of nadolol concentrations in plasma to check compliance or to monitor accumulation of the drug in patients with diminished renal clearance.

Materials and Methods

Reagents. All reagents were of analytical grade. Solvents had been distilled in glass by the supplier (Caledon Laboratories Ltd., Georgetown, Ontario L7G 4R9, Canada). Deionized water was distilled in an all-glass still.

Standards. A 1 g/L stock solution of nadolol was prepared by dissolving 100 mg of nadolol in 100 mL of methanol. This solution was stable for three months when stored at 4 °C. The 400 µg/L plasma-matrix drug standard was prepared by diluting 40 µL of nadolol stock to 100 mL with blood-bank plasma; additional plasma standards of 200, 100, 50, and 25 µg/L were prepared by appropriate dilution with plasma. These standards were stable for six months when stored at −15 °C in 2-mL portions. The stock 1 g/L internal standard solution was prepared by dissolving 100 mg of SQ 23554 in 100 mL of methanol. This solution was stable for three months at 4 °C. For the working solution of internal standard, 10 µL of stock solution was diluted to 10 mL with 0.1 mol/L sodium acetate when required.

Specimen collection. Blood was collected from patients who had received nadolol in a constant daily dosage (80, 160, or 320 mg) for at least 14 days. Specimens were collected at four 2-h intervals in blue-capped heparinized Vacutainer Tubes (Becton Dickinson, Orangeburg, NY 10962). The tubes were centrifuged within 2 h of blood collection, and the plasma was collected with Pasteur pipettes and stored in disposable plastic tubes at −15 °C until analyzed.

Sample preparation. "BondElut C18" 1-mL disposable extraction columns and a "VacElut" system were obtained from Analytichem International Inc., Harbor City, CA 90710. The columns were washed under suction twice with methanol, once with 0.1 mol/L sodium acetate, and twice with water. Working internal standard (100 µL) and plasma sample (1.0 mL) were applied to the washed columns. Suction was adjusted so that the liquid passed through the columns in 30–50 s. The columns were washed twice with water, once with methanol/water (30/70 by vol), and finally with 0.5 mL of pentane. The columns were transferred to labeled 16 × 100 mm disposable glass tubes. We added 1 mL of methanol to each column and centrifuged the tubes to elute the columns. The eluate was evaporated under a stream of nitrogen at 40 °C. The residue in each tube was dissolved in 100 µL of mobile phase, and 10 µL was injected into the chromatograph.

Chromatography. The chromatographic separation was performed isocratically at room temperature with a dual-piston reciprocating pump (Model 100A; Beckman Instrument Co., Toronto, Ontario M5Z 5T2). Injectors are made with a syringe-loading injector with a 20-µL loop (Model 7125; Rheodyne, Cotati, CA 94928). The peaks are detected with a fluorescence detector (Model RF-530; Shimadzu Scientific Instrument Inc., Columbia, MD 21045) at $\lambda_{ex} = 265$ nm and $\lambda_{em} = 305$ nm. We use a 15 cm × 4.1 mm PRP-1 column.

\[ HO \]
\[ \text{O} - \text{CH}_2 - \text{CH} - \text{CH}_2 - \text{NH}_2 + \text{R} \]

\[ \text{HO} - \text{CH}_2 - \text{CH} - \text{CH}_2 - \text{NH}_2 + \text{R} \]

1. $\text{R} = \text{C} (\text{CH}_3)_{\text{g}}$

2. $\text{R} = \text{(CH}_2)_2 - \text{C}_3$

Fig. 1. Structural formulas of (1) nadolol and (2) SQ 23554

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Results and Discussion
Nadolol absorbs weakly at 280 nm, where it is difficult to detect in concentrations less than 100 μg/L. Detection at 205 nm provides adequate sensitivity, but the many extraneous peaks in chromatograms of plasma extracts obtained with this solid-phase extraction again makes difficult the determination of <100 μg of nadolol per liter of plasma. Using the Schotten–Bauman reaction, we obtained a good yield of benzyl derivative of nadolol with high absorbance at 240 nm. However, on chromatography, the benzyl derivative of nadolol gave a double peak and therefore could not be used. Nadolol oxidized with periodate produces a dialdehyde (2), which we trapped in acidic medium as the 2,4-dinitrophenylhydrazone. This derivative also had high absorbance at 350 nm, but separating it from extraneous impurities proved difficult.

Determination of nadolol in serum by liquid chromatography with electrochemical detection has been reported (4). Nadolol has recently been measured by thin-layer chromatography by detection of its native fluorescence (5). Detection of native fluorescence of nadolol in the flow cell of the detector allows sensitive, specific detection of the drug and the internal standard in the extracts of plasma. Pindolol (6) and pranolol (7), which have some of the structural features of nadolol, have been measured in plasma by fluorescence detection at λex = 220 nm. Because the xenon lamp of our fluorescence detector does not allow excitation at less than 260 nm, we cannot comment as to whether the sensitivity or specificity of detection would improve at lower wavelength of excitation. Figure 2 shows chromatograms of an extract of drug-free plasma and of plasma supplemented with nadolol, final concentration 100 μg/L. Only a few extraneous peaks appear and there is no interference from any possible peak from a previous injection, even after 20 injections of plasma extracts. The peaks for nadolol and the internal standard are symmetrical and clearly resolved. The ratio of peak areas or of peak heights of nadolol/internal standard is linearly related to nadolol concentration over the range 10–400 μg/L, and the standard curve passes through the origin. The procedure allows quantitative determination of as little as 10 μg of nadolol per liter. The sensitivity of the procedure may be further increased by dissolving the dried residue in a smaller volume of mobile phase (<100 μL).

Because of the presence of hydroxyl groups, nadolol is quite polar. Its extraction from plasma for GC-MS is tedious, involving lyophilization of the acidic aqueous extract obtained by back-extracting the butyl acetate extract of plasma. The present extraction procedure is convenient, involves a minimum of glassware, and has a 85–95% yield. The analytical recovery of the drug is lessened if the mixture of plasma and internal standard passes through the column in less than 30 s. There is no change in the ratio of drug to internal standard after extracting an aqueous mixture of the two compounds by the present procedure. SQ 23554, being an isomer of nadolol, is an ideal internal standard for this determination. The plasma extract obtained by the present procedure is also suitable for analysis by GC with the use of a nitrogen-selective detector (8).

Analysis of plasma supplemented with 100 μg of nadolol per liter showed a within-batch CV of 5.3% (n = 11, mean = 100.9 μg/L) and a between-batch CV of 8.3% (n = 10, mean = 100.6 μg/L).

Figure 3 summarizes our results for analysis of plasma samples from patients receiving different doses of nadolol.

![Figure 2](image-url)  
Fig. 2. Liquid chromatograms of (left) drug-free plasma, (right) plasma with 100 μg/L added nadolol  
Detector: sensitivity, high; attenuation, 8; recorder, 20 mV, 5 min/cm. f = nadolol; 2 = SQ 23554 (internal standard)

![Figure 3](image-url)  
Fig. 3. Nadolol concentrations in plasma from patients being treated with it.
Even with only a few patients at each dosage, it can be seen that this procedure allows good discrimination between doses. (Because of the small number of patients studied, we did not calculate confidence intervals for each mean value.) Some of the patients were also receiving hydrochlorothiazide and hydralazine, these and other commonly prescribed antihypertensive drugs do not interfere with this assay for nodol.

We conclude that this simple chromatographic procedure is suitable for routine determination of therapeutic concentrations of nodol in plasma.

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Improved Determination of Aluminum in Serum by Electrothermal Atomic Absorption Spectrophotometry

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This method for determining aluminum in human serum involves electrothermal atomic absorption spectrophotometry. A longer drying time allows less pre-dilution of the sample, and the method also includes a flush cycle after atomization. Standard-addition methodology is used to eliminate matrix effects and the need for a standard curve. We used this method on serum from 50 normal persons and from 34 patients with chronic renal failure who were on long-term intermittent hemodialysis. The mean normal serum aluminum concentration was 6.1 μg/L (CV 2.7%), and values for the patients ranged from 13 to 475 μg/L.

Additional Keyphrases: reference interval · trace elements

The biochemistry and toxicology of aluminum has been intensely investigated since its etiological role in disorders such as dialysis encephalopathy, osteomalacia, and Alzheimer's disease was suggested over 10 years ago (1–2). Several studies have attempted to define the normal serum aluminum concentration. Because of inadequate attention to sample contamination and other problems, published values for normal serum aluminum concentration vary widely (3).

Among the methods available for determining aluminum in serum, electrothermal (furnace) atomic absorption spectrophotometry seems to offer the best combination of sensitivity, simplicity, and low cost. Several methods have recently been published for determining aluminum in serum by this technique (4–7), and most of them share the common features of meticulous attention to avoiding contamination, good sensitivity, a two-stage ashing cycle, and some prior dilution of the sample serum. However, in our hands these methods, unmodified, are unacceptably imprecise. The precision we observed was due, we believe, to either incomplete or too-vigorous drying stages, resulting in wide variations in sequential absorbance measurements on a single sample, or matrix interferences that invalidate comparison with an aqueous standard curve. Accordingly, we modified the method of Gardiner et al. (4), seeking to develop a method that is largely independent of matrix variations and is also reproducible. We describe the resulting sensitive, precise, and rugged method for routine measurement of aluminum in serum, and report a normal reference interval.

Materials and Methods

Instrumentation: For these analyses we used a Model 5000 atomic absorption spectrophotometer with a Model 500 heated graphite atomizer and an AS40 autosampler (all from Perkin-Elmer Corp., Norwalk, CT 06856). Instrument settings and furnace program are detailed in Table 1.

Reagents: Standards were prepared by appropriate dilution of a 1 mg/mL (1000 ppm) Certified Aluminum Atomic Absorption Standard (Fisher Scientific, Pittsburgh, PA 15219) with distilled water.

Specimen collection: Venous blood was sampled from 50 healthy volunteers, ages 18 to 50 years, with equal numbers of men and women. The specimens were collected into a plastic syringe through a plastic catheter (Quik-Cath; Travensol Laboratories, Inc., Deerfield, IL 60015; or Deseret Angiocath; The Deseret Co., Sandy, UT 84070). The blood