Determination of Plasma Procainamide and \( N \)-Acetylprocainamide Concentration by High-Pressure Liquid Chromatography

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We describe a routine method for determining concentrations of the antiarrhythmic drug procainamide and its active metabolite, \( N \)-acetylprocainamide, in plasma. A simple extraction of 1.0 ml of plasma is followed by separation and chromatographic analysis by use of a column containing microparticulate silica. \( p \)-Nitro-\( N \)-(2-diethylaminoethyl)benzamide hydrochloride was synthesized and used as the internal standard. Total chromatographic time is only 7 min. The day-to-day CV during three months of daily use was less than 4\% of the mean for each compound, and we saw no deterioration in column performance during this time. Phenobarbital, phenytoin, lidocaine, primidone, methsuximide, quinidine, and their metabolites do not interfere.

Procainamide (PA) is an antiarrhythmic drug that has been commonly used for over two decades. Because of individual variations in gastrointestinal absorption, metabolism, and excretion of PA (1-4), the correlation between dose and therapeutic and toxic effects is poor. Thus its measurement in plasma is advocated as a useful, if not mandatory, guide to therapy. The accepted therapeutic concentration range for PA is 4–8 mg/liter (1), but its major metabolite, \( N \)-acetylprocainamide (NAPA), which also has an antiarrhythmic effect in humans (5), is found in similar concentrations in the plasma of patients who are undergoing therapy with PA (2, 3). Therefore, therapy can be optimal only if both PA and NAPA are considered. Recently, Atkinson et al. (6) recommended that the sum of PA and NAPA concentrations should be at least 5 mg/liter, and not exceed 20–30 mg/liter.

The techniques most commonly used for PA analysis are spectrophotometry (7) and spectrofluorometry (1), both of which lack specificity and are subject to some interference by NAPA. Matusik and Gibson (8) recently described the fluorometric determination of both PA and NAPA, with a coefficient of variation (CV) of 10\% for each compound. Gas-chromatographic procedures have the specificity which the spectral procedures lack. Elson et al. (3) have simultaneously measured both PA and NAPA with a precision of 4\% and 5\%, respectively. Frisild et al. (9) also determined both by gas chromatography, with a CV of 3\% for PA and 8\% for NAPA. Our experience is that gas-chromatographic procedures are not very sensitive at low concentrations (especially for NAPA). Special small-bore columns are needed to measure NAPA in concentrations of less than about 5 mg/liter, and the useful life of these columns is relatively short. Also, evacuated blood-collection tubes cannot be used, because a substance in their stoppers interferes with the assay (11). Reidenberg et al. (4) reported a thin-layer chromatographic method with CV's of 9\% for PA and 11\% for NAPA, but this procedure is inconvenient for use in routine laboratory determination.

High-pressure liquid chromatography (HPLC) offers several advantages as an analytical tool, most importantly specificity, reproducibility, speed, and the ability to detect heat-labile compounds. Recently, several such methods for PA and NAPA analysis by reversed-phase chromatography have been published. That of Carr et al. (10) gives a CV of 4\% for both compounds in the range of 4–8 mg/liter (analysis time after injection, 16 min). Sample preparation involves a 5-min extraction period and the use of plastic tubes and silanized glassware. The internal standard, \( N \)-formylprocainamide, hydrolyzes to PA in aqueous solution at room temperature, so it must be kept frozen. Shukur et al. (12) used an external standard method that gives approximately 55\% extraction efficiencies after a somewhat laborious procedure. The overall precision of the assay is less than for internal standard procedures. Rocco et al. (13) also used reversed-phase high-performance liquid chromatography in a procedure that is rapid and gives within-run coefficients of variation of 4\% for PA and 2\% for NAPA.

We describe here a normal phase high-performance liquid chromatographic method for determining PA and NAPA in plasma in the routine laboratory by use of an inexpensive, fixed-wavelength chromatograph and a microparticulate silica column. Analysis time after a simple extraction is only 7 min.
and column life and day-to-day reproducibility are very good.

Materials and Methods

Apparatus. For analysis we used a Model 848 liquid chromatograph (du Pont Instruments, Wilmington, Del. 19898), equipped with a 254-nm ultraviolet detector and fitted with a Rheodyne Model 70-10 sample injection valve (20-μl sample loop) and a Zorbax-SIL silica column (2.1 mm × 25 cm, du Pont). The mobile phase consisted of 100 ml of methanol, 2 ml of water, and 0.1 ml of morpholine (Aldrich Chemical Co., Inc., Milwaukee, Wis. 53233). Flow rate was 1.9 ml/min at a pressure of 13.8 MPa (2000 psi). Ultraviolet spectra were recorded with an Aminco DW-2 UV-VIS spectrophotometer (American Instrument Co., Silver Spring, Md. 20910).

Standards. Procaainamide hydrochloride (Figure 1, 1), mp 167-169 °C, was isolated by evaporation of Pronestyl solution for injection (Squibb) under reduced pressure and recrystallization of the residue from 2-propanol.

We prepared N-acetyl procainamide (Figure 1, 2), mp 138-140 °C, by reacting an aqueous solution of PA-HCl with acetic anhydride. Neutralization and extraction with methane chloride gave the free base, which was recrystallized from ethyl acetate. Aqueous solutions containing 1, 3, 5, 7, 10, 15, and 20 mg each of PA and NAPA per liter were prepared for use as analytical standards.

The internal standard, p-nitro-N-(2-diethylaminoethyl)benzamide hydrochloride (Figure 1, 3), mp 167-169 °C, was synthesized by reacting N,N-diethylethlenediamine (Aldrich) with the acid chloride of p-nitrobenzoic acid (prepared by using thionyl chloride) in chloroform. The organic solution was evaporated and the residue was recrystallized twice from ethanol. An aqueous solution (5.00 mg/liter) was used as internal standard.

All these standard solutions are stable at room temperature for several months.

Procedure. Separate plasma from heparinized blood samples by centrifugation and either store at −17 °C or analyze directly as follows. Add 1 ml of plasma, 1 ml of internal standard solution, 0.2 ml of sodium hydroxide (2 mol/liter), and 5 ml of ethyl acetate to a 10-ml glass-stoppered centrifuge tube and shake the mixture for 30 s. After centrifugation, transfer the organic phase to a 10-ml pear-shaped flask and evaporate under reduced pressure at room temperature. Dissolve in 100 μl of the mobile phase and inject a portion into the chromatograph. Calculate the peak-height ratios of PA/internal standard and NAPA/internal standard.

Fig. 1. Structural formulas for 1, procainamide hydrochloride, 2, N-acetylprocainamide, and 3, p-nitro-N-(2-diethylaminoethyl)benzamide hydrochloride

Fig. 2. Chromatogram of an extract of 1.0 ml of plasma from a patient receiving PA
The specimen contained 5.1 mg of PA, 5.4 mg of NAPA and 5 mg of p-nitro-N-(2-diethylaminoethyl)benzamide hydrochloride (as internal standard) per liter

Extraction efficiency was determined by the above procedure, modified as follows: 1 ml of water was substituted for the internal standard solution, and the organic phase was discarded after extraction. Internal standard, 5 μg in 50 μl of water, was added, and the aqueous solution re-extracted with ethyl acetate. The organic phase was then evaporated and analyzed by high-performance liquid chromatography.

Results and Discussion

Although many solvents and solvent mixtures gave high extraction efficiencies, we selected ethyl acetate over other solvent systems because of its relative density (<1) and volatility. Efficiency of extraction of plasma solutions containing 3 or 20 mg of each drug per liter exceeded 92% for PA and 87% for NAPA at each of the two concentrations.

Aqueous standards were analyzed, and from these data standard curves were constructed by plotting peak-height ratio (drug/internal standard, y-axis) vs. drug concentration (mg/liter) as the base free. Each point on the curve was the mean of at least 10 determinations. The curves were linear for both compounds (PA: r = 0.9998, slope = 0.066, y-intercept = −0.04; NAPA: r = 0.9992, slope = 0.215, y-intercept = −0.08) with slightly negative intercepts that represent a slight curvature for concentrations below 3 mg/liter. Standards prepared in plasma gave identical results. The detection limits for PA and NAPA were 0.1 and 0.01 mg/liter, respectively.

Figure 2 depicts a chromatogram of an extract of patient's
plasma that contained, per liter, 5.1 mg of PA and 5.4 mg of NAPA. Although the molar absorptivities at \( \lambda_{\text{max}} \) for each compound are similar, the detector is less sensitive to PA because its \( \lambda_{\text{max}} \) (283 nm) is further from the detection wave length than is the internal standard (265 nm) or NAPA (269 nm). Monitoring the column effluent at 285 nm would increase the sensitivity for PA by a factor of 2.3, but this is not necessary for PA concentrations in the therapeutic range. Furthermore, the low price of the 254-nm fixed-wavelength chromatograph makes it especially attractive for use in a routine drug analysis laboratory.

For routine analysis, relatively constant chromatographic characteristics and good day-to-day reproducibility are extremely important. This procedure satisfies both of these requirements. We have used the same column for more than four months (over 400 patients' samples) with no change in flow rate, resolution, or peak shape. Daily analysis of a 5 mg/liter standard for 10 weeks gave a mean value of 5.07 mg/liter (SD, 0.23; CV, 4.5%) for PA and 4.88 \( \mu \)g/liter (SD, CV, 0.14; 2.8%) for NAPA.

Analysis of plasma extracts from patients receiving phenobarbital phenytoin, lidocaine, primidone, or methsuximide showed that these drugs or their metabolites did not interfere with the analysis, nor did extracts of aqueous caffeine solutions. Although quinidine, in this system, has a retention time of 5.0 min, it does not add to the peak heights of either PA or NAPA. Analysis of plasma samples from patients undergoing therapy with quinidine showed another small peak (sometimes two poorly resolved peaks) with a retention time of 4.1 min. Judging from our analysis of six patients' samples (quinidine >3 mg/liter), this presumed metabolite of quinidine would cause the overestimation of NAPA by at most 0.2 mg/liter.

A comparison of data obtained by the gas-chromatographic procedure of Elson et al. (3) and this method indicated that the present analysis is superior for monitoring PA and NAPA in the blood. Although the data from this comparison are not complete enough for publication, there is clear evidence that the present analysis is more accurate and reproducible for concentrations below 5 mg/liter, for either compound.

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References