Modified Colorimetric Method for Procainamide in Plasma

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We describe a modified colorimetric method for determining procainamide in plasma by use of a diazotization and coupling reaction with N-(1-naphthyl)-ethylene-diamine dihydrochloride (Marshall's reagent). The sensitivity of the assay is increased twofold over the originally reported colorimetric method, and the lower limit of detection for the assay of a 1.0-ml plasma sample has been decreased to 0.5 mg/liter.

The original colorimetric assay for plasma procainamide, which utilized a diazotization step in the production of a colored product for spectrophotometric analysis (1), is characterized only between 5 and 40 mg/liter (2). In our hands, the assay as originally described gave highly variable results below 5 mg of procainamide per liter. A fluorometric assay for this drug is not specific, in that N-acetylprocainamide, a metabolite of the drug, interferes in the analysis (3). Significant concentrations of N-acetylprocainamide in plasma can result after doses of procainamide to man (4, 5). The reproducibility or precision of the fluorometric method is not indicated. However, a recently reported fluorometric method for both procainamide and its N-acetyl metabolite appears to have overcome these difficulties (6). A gas–liquid chromatographic method has also been reported for procainamide (7), but we encountered inadequate reproducibility of this method at concentrations in plasma of less than 4 mg/liter.

We have modified the colorimetric method of Mark et al. (2) to develop an assay for procainamide that can be used to investigate the disposition of this drug in patients with chronic renal failure, as it has been recently demonstrated that good agreement exists between the colorimetric and gas–liquid chromatographic methods for determination of this drug (8). Here, we report the results of this modified assay, and compare them with results by the original colorimetric method (2).

Materials and Methods

Reagents. Procainamide hydrochloride was supplied by E. R. Squibb and Sons Ltd., Montreal, P.Q. N-Acetylprocainamide hydrochloride was supplied by Dr. D. Drayer (Temple University, Philadelphia, Pa.). Both chemicals were used as received. Hydrochloric acid ("Baker Analyzed Reagent") was obtained from Canadian Laboratory Supplies Ltd., Montreal, P.Q. Sodium nitrite, sodium hydroxide, and sodium chloride ("Analar" grade) were obtained from British Drug Houses Ltd., Montreal, P.Q. Ammonium sulfamate ("Certified") and N-(1-naphthyl)-ethylene-diamine dihydrochloride (Marshall's reagent; N-30) were obtained from Fisher Scientific Co., Montreal, P.Q. All chemicals were dissolved in doubly distilled water.

Solutions. Hydrochloric acid (1 mol/liter), sodium hydroxide (5 mol/liter), sodium nitrite (1 g/liter), ammonium sulfamate (5 g/liter), and Marshall's reagent were stored in glass containers at 2–4 °C until used. Procainamide hydrochloride (5.77 g/liter) was dissolved in distilled water to give a stock solution of 5 g/liter with respect to procainamide. The nitrite and sulfamate solutions were stable for at least two weeks. All other solutions were stable for at least one month. Prepared plasma standards kept frozen at −20 °C were stable for at least three months.

Procainamide assay. Pipette 1.0 ml of plasma into a 12-ml test tube containing 0.25 g of sodium chloride, 0.2 ml of sodium hydroxide, and 0.5 ml of water. Stir the contents on a vortex-type mixer. Pipette 10 ml of dichloromethane into the tube. Cap the tube and shake it for 20 min. Centrifuge the tube (10 min, 1000 × g), remove 8.0 ml of the organic (lower) layer, and evaporate it at 40 °C under a stream of nitrogen (we used a Brinkman SC/48 Sample Concentrator; Brinkman Instruments Canada Ltd., Toronto, Ont.). Place the containers in an ice bath, add 2.0 ml of hydrochloric acid, mix the contents with a vortex-type mixer, and return the container to the ice bath for 10 min. Add 1.0 ml of the ammonium sulfamate solution, mix, and add 1.0 ml of Marshall's reagent. Remove the sample from the ice bath, stir, and allow to stand at room temperature for 15 min. Determine the absorbance of the solution vs. a plasma blank at 550 nm.

Calibration curve. Pipette procainamide standard solutions into a known volume of plasma to give a solution that contains 25 mg of procainamide per liter. Use this plasma standard to prepare other plasma standards that contain...
from 0.5 to 25 mg procainamide per liter. Analyze these standards in duplicate as described above.

Statistics. The calibration-curve data were analyzed by a least-squares regression method. This regression analysis was then subjected to analysis of variance, and the 95% confidence limits were determined (9).

**Results and Discussion**

Figure 1 shows a representative calibration curve, with its 95% confidence limits, derived from data generated by the method just described. Although the curve represents only concentrations to 10 mg/liter procainamide, the slope and accuracy are the same from 0.5 to 25 mg/liter (Table 1). Repetition of this assay on 10 separate days between the concentrations of 0.5 to 25 mg of procainamide per liter gave a slope of 0.0343 ± 0.0006 (mean ± SE). Repetitive analysis of a 5 mg/liter standard resulted in a mean (± SE) concentration of 4.97 ± 0.07 mg/liter for procainamide (n = 10). These determinations were made over a six-month period.

Although mean values are indicated for each concentration in Figure 1, all values were used in the determination of variance and calculation of confidence limits. The analysis indicated a significant regression with insignificant variation owing to multiple determinations for any given drug concentration. Therapeutic concentrations of lidocaine, digoxin, hydrochlorothiazide, triamterene, furosemide, or warfarin in plasma do not interfere. Whole blood, plasma, or erythrocytes from the blood bank gave no absorbance at 550 nm; i.e., we have found no interference with this assay attributable to storage of blood samples or to endogenous components of blood. However, one must bear in mind that Marshall's reagent can react with other primary aromatic amines that might be present, such as sulfonamides. We have not encountered such problems commonly. Added N-acetylprocainamide did not alter results for procainamide in plasma by this method. Hemolyzed or lipemic samples did not interfere with the assay. Either plasma, packed erythrocytes, or whole blood could be used as the sample; all give similar values. The least variance was encountered with plasma samples.

Table 1 compares our assay and that of Mark et al. (2). Analytical recovery is quantitative for both methods, but variance is less and sensitivity greater by the modified procedure we report here.

One of the most important factors affecting consistency in this assay is temperature control (0°–2°C) during the diazotization procedure. Use of an ice bath to stabilize the diazo intermediate until the coupling dye is added is essential. It results in increased coupling owing to stabilization of the intermediate and also prevents hydrolysis of N-acetylprocainamide back to procainamide in the acid medium. The increased sensitivity of our method allows more accurate determinations of procainamide concentrations of less than 4 mg/liter; this is important for accurate investigation of the kinetic disposition of the drug after a therapeutic dose. The smaller blood sample required for these determinations in renal-failure patients is important. The assay requires less solvent for the extraction step, so there is a substantial cost-saving when many assays are required. We estimate that it is possible to derive a calibration curve and analyze more than 40 samples per day in duplicate by this method.

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**References**