Simultaneous Quantitation of Quinidine, Procarainamide, and N-Acetylprocainamide in Serum by Gas–Liquid Chromatography with a Nitrogen–Phosphorus Selective Detector

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We describe a single-run method for quantitating quinidine, procarainamide, and N-acetylprocainamide, involving gas–liquid chromatography with a nitrogen–phosphorus selective detector. Within-run precision (CV) was 3% (x̄ = 2 mg/L, n = 20), 6.9% (x̄ = 4 mg/L, n = 10), and 1.5% (x̄ = 8 mg/L, n = 8) for procarainide; 7.7% (x̄ = 4 mg/L, n = 14), 1.6% (x̄ = 8 mg/L, n = 16), and 2.3% (x̄ = 12 mg/L, n = 12) for procarainamide; and 6.3% (x̄ = 5 mg/L, n = 6), 3.6% (x̄ = 10 mg/L, n = 20), and 4.0% (x̄ = 20 mg/L, n = 10) for N-acetylprocainamide. Between-run precision was 3.0% (x̄ = 2 mg/L, n = 20), 7.0% (x̄ = 4 mg/L, n = 9), and 2.8% (x̄ = 8 mg/L, n = 9) for quinidine; 4.7% (x̄ = 4 mg/L, n = 10), 3.3% (x̄ = 8 mg/L, n = 20), and 1.9% (x̄ = 12 mg/L, n = 10) for procarainamide; and 9.3% (x̄ = 5 mg/L, n = 6), 4.3% (x̄ = 10 mg/L, n = 20), and 3.8% (x̄ = 20 mg/L, n = 10) for N-acetylprocainamide. Tube stoppers that contain a rubber plasticizer interfere with the technique. Clinical application and correlation with drug concentrations by this technique are discussed.

Additional Keyphrases: drug assay · antiarrhythmic drugs · analytical error · monitoring therapy

Quinidine, a widely used antiarrhythmic agent, is most often administered orally for control of atrial and ventricular arrhythmias. A weak base, it is primarily metabolized by the liver, has an average biological half-life of 6 h in healthy individuals, has a large volume of distribution, and is highly (80–90%) bound to plasma proteins (1, 2). The therapeutic concentration range appears to be 2.3–5.0 mg/L (1).

Procarainamide is most often administered orally for control of chronic ventricular arrhythmias (2, 3). Recently, it has also been used intravenously for control of ventricular ectopy in acute ischemia (4). In contrast to quinidine, procarainamide has a shorter half-life (3–4 h), is weakly (14–23%) bound to plasma proteins, and has a major renal route of elimination. The therapeutic range is from 3 to 8 or 10 mg/L. Procarainamide is also metabolized in the liver to N-acetylprocainamide, a compound with antiarrhythmic activity and a renal elimination route (5).

The role of N-acetylprocainamide in therapeutics is currently under investigation; however, it is already clear that measurements of concentrations in serum are clinically indicated.

These three drugs have been quantitated by spectrophotofluorometry (7, 8), gas chromatography with electron-capture detector (9, 10), and “high-pressure” liquid chromatography (11). Previous methodology rarely dealt with all three compounds (12). However, the concentrations of any of them in plasma are frequently quantitated in clinical practice; moreover, these drugs are occasionally co-administered to the same patient. Because their separate determination is inconvenient and time consuming, whether for clinical or research purposes, we developed a method for their simultaneous determination in serum by gas chromatography, with use of a nitrogen–phosphorus selective detector.

Materials and Methods

We used a Model 5710A gas chromatograph, a nitrogen–phosphorus selective detector, and a Model 3380A integrator (Hewlett-Packard, Avondale, PA 19311). The coiled glass column was 2 mm (i.d.) × 1.83 m, packed with 3% OV 17, 100–120 mesh on Gas Chrom Q (Applied Science Labs, Inc., State College, PA 16801). Reagents included: sodium hydroxide (analytical grade), 50 g/L dichloromethane ("nongrade"); and ethyl acetate ("nongrade"), all from Mallinckrodt, Inc., St. Louis, MO 63147. Blood was collected into glass tubes or Vacutainer Tubes (Becton Dickinson and Co., Rutherford, NJ 07070), without plasticizer.

Standards: All standards were dissolved in doubly distilled water. The internal standard was the dipropyl analog of procarainide (E. R. Squibb & Sons, Inc., Princeton, NJ 08640) at 20 g/L. Stock solutions of quinidine sulfate, 50 mg/L, and procarainamide hydrochloride, 40 mg/L, both from Sigma Chemical Co., St. Louis, MO 63178, and N-acetylprocainamide (Squibb & Sons, Inc.), 40 mg/L, were stored at 4 °C. These standards and reagents were stable for at least six months.

Operating conditions: We used nitrogen as the carrier gas with a flow rate of 40 mL/min. The injector-port temperature was 300 °C. The detector temperature was 350 °C with an air flow to the detector of 60 mL/min and a hydrogen flow rate of 3.5 mL/min. The oven temperature was programmed to start at 230 °C for 4 min, then increase at 16 °C/min to a maximum temperature of 280 °C for 4 min. We applied 15.8 V to the detector. Careful attention to voltages and avoidance of fluctuations in line current are necessary for accurate quantitation.

Procedure: Rinse acid-washed glassware with de-ionized distilled water before use. Add 0.5 mL of serum to a 12-mL glass centrifuge tube and adjust to alkaline pH with 0.25 mL of a 50 g/L NaOH solution. Add 40 μL of the internal standard (the dipropyl derivative of procainamide), vortex-mix, add 2 mL of methylene chloride, and mix again for 2 min. After centrifugation (600 X g, 5 min, room temperature), transfer the bottom layer to a clean glass tube and extract it again with methylene chloride. Evaporate the bottom layer in a stream of nitrogen and reconstitute the residue in 25 μL of ethyl acetate. Inject 4 μL into the injection port. With our apparatus, calculation was automatic with the integrator.

Results

Figure 1 shows chromatograms from a serum blank, a
serum sample containing internal standard and all three reference drugs, and serum from a typical patient receiving both quinidine and procainamide. Serum blanks gave no interfering peaks except as described for blood collected in evacuated rubber-stoppered tubes containing tris(2-butoxyethyl)phosphate as described below. Fortified sera representing subtherapeutic, therapeutic, and toxic concentration ranges (per liter: 1, 2, 4, 8, and 15 mg of quinidine sulfate; 2, 4, 8, 12, and 20 mg of procainamide; and 5, 10, 20, 30, and 40 mg of N-acetyprocainamide) were each analyzed in triplicate on three separate days. Additional samples to quantitate within and between-run precisions and analytical recoveries were included.

Quinidine: Plotting the quinidine concentration against the ratio of the integrated area of internal standard to that of quinidine, we obtained a slope of 0.34 (SD 0.01) and a y-intercept of 0.07 (SD 0.08) for three successive days, a standard error of the estimate (S_yx) of 0.15, and a correlation coefficient of 0.99. Analytical recovery was 97 (SD 8) % at 2 mg/L (n = 3), 91 (SD 2) % at 4 mg/L (n = 5), and 98 (SD 1) % at 15 mg/L (n = 3). There was no interference with quinidine quantitation at 2, 4, or 15 mg/L in the presence of procainamide (6 mg/L) or N-acetyprocainamide (10 mg/L). Within-run precision (CV) was 3% (X = 2 mg/L, n = 20), 6.9% (X = 4 mg/L, n = 10), and 1.5% (X = 8 mg/L, n = 8); between-run precision for the same samples was 3.0% (n = 20), 7.0% (n = 9), and 2.8% (n = 9), respectively.

Procainamide: Plotting the procainamide concentration against the ratio of the areas of internal standard to procainamide yielded a slope of 0.85 (SD 0.01) and a y-intercept of -0.23 (SD 0.17) for three successive days, a standard error of the estimate of 0.27, and correlation coefficient of 0.99. Analytical recovery was 110 (SD 6) % at 2 mg/L (n = 3), 110 (SD 1) % at 8 mg/L (n = 3), and 108 (SD 4) % at 20 mg/L (n = 3). There was no interference with procainamide quantitation at 2, 8, or 20 mg/L in the presence of quinidine (8 mg/L). Procainamide concentrations were falsely increased in the presence of N-acetyprocainamide by 5% of the N-acetyprocainamide concentration. Because the two peaks elute at different times, this suggests a breakdown of N-acetyprocainamide to procainamide, thus giving a small interference in quantitating procainamide. Within-run precision was 7.7% (X = 4 mg/L, n = 14), 1.6% (X = 8 mg/L, n = 16), and 2.3% (X = 12 mg/L, n = 12); at the same concentrations, between-run precision was 4.7% (n = 10), 3.3% (n = 20), and 1.9% (n = 10), respectively.

N-Acetyprocainamide:Plotting the concentration vs the ratio of area of internal standard to area of N-acetyprocainamide for assays on three successive days gave a slope of 0.59 (SD 0.03) and a y-intercept of 0.28 (SD 0.17), a standard error of the estimate of 0.19, and a correlation coefficient of 0.99. Analytical recovery was 101 (SD 8) % at 5 mg/L (n = 6) and 113 (SD 4) % at 20 mg/L (n = 3). There was no interference with N-acetyprocainamide quantitation at 2, 10, or 20 mg/L in the presence of quinidine (8 mg/L) or procainamide (8 mg/L). Within-run precision was 6.3% (X = 5 mg/L, n = 6), 3.6% (X = 10 mg/L, n = 20), and 4.0% (X = 20 mg/L, n = 10). Between-run precision at the same concentration was, respectively, 9.3% (n = 6), 4.3% (n = 20), and 3.8% (n = 10).

Blood-collection methods: Sera from blood collected in previously distributed Vacutainer Tubes with rubber stoppers that contained the plasticizer tris(2-butoxyethyl)phosphate gave an early peak, which interfered with procainamide quantitation. Overestimation of integrated procainamide area led to subsequent overestimation of drug concentration. Table 1 compares results from our current method with an enzyme immunoassay method (EMIT-cad; Syva Co., Palo Alto, CA 94304) for five sera containing procainamide and N-acetyprocainamide. Blood from patient C, obtained in a Vacutainer Tube contaminated with plasticizer, was overestimated for procainamide concentration by the current method. Such interference is not seen in sera from blood collected in the more recently distributed rubber-stoppered Vacutainer Tubes, which no longer contain tris(butoxyethyl)phosphate.

Clinical data: The method described here has produced satisfactory results for us in general and in specialized clinical and research settings, such as close follow-up of patients who have been resuscitated from prehospital cardiac arrest (13). In these patients “therapeutic” concentrations of the membrane-active antiarrhythmic agents procainamide or quinidine may protect against recurrence of cardiac arrest. Concentrations of these drugs in the blood of these patients are followed routinely and demonstrate a large degree of intra- and inter-individual variability (Figure 2), underscoring the utility of periodic determinations of plasma drug concentrations.

We have also used this method to quantitate total and unbound quinidine in the determination of the free drug fraction (14). The mean unbound quinidine fraction was 8.1 (SD 3.2) % (n = 36) in outpatients with cardiac disease. Inpatients with cardiac disease had an mean unbound fraction of quinidine of 6.3 (SD 4.6) % (n = 101), procainamide 62.3 (SD 11.2) % (n = 92), and N-acetyprocainamide 70.0 (SD 14.4) % (n = 15).

We compared the present method with another standard clinical assay, to ascertain that the ranges of results were similar and that no bias existed that would influence the clinical interpretation of the quantitative results. Fifty clinical

Table 1. Comparison of Procainamide (PA) and N-Acetyprocainamide (NAPA) Concentrations (mg/L) Determined by Gas Chromatography and EMIT

<table>
<thead>
<tr>
<th>Sample</th>
<th>Gas Chromatography</th>
<th>EMIT</th>
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<tbody>
<tr>
<td></td>
<td>PA</td>
<td>NAPA</td>
</tr>
<tr>
<td>Serum + PA (3 mg/L) + NAPA</td>
<td>3.3</td>
<td>11.7</td>
</tr>
<tr>
<td>Serum + PA (11 mg/L) + NAPA</td>
<td>10.8</td>
<td>3.0</td>
</tr>
<tr>
<td>Serum + PA (3 mg/L) + NAPA</td>
<td>4.9</td>
<td>2.0</td>
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* Blood sample collected in "old" rubber-stoppered Vacutainer Tube containing tris(2-butoxyethyl)phosphate. See text.
blood specimens with procainamide concentrations of 0 to 13.7 mg/L and associated N-acetylprocainamide values ranging from 0 to 14.7 mg/L were quantitated by both the current method and the EMIT assay method. The mean procainamide and the N-acetylprocainamide concentrations by our current method were 4.8 (SD 3.2) and 5.4 (SD 3.6) mg/L, respectively; by EMIT, these values were 4.9 (SD 3.0) and 5.4 (SD 3.4) mg/L, respectively. There was a significant correlation between results by the two methods \( r = 0.95, p < 0.01 \) for procainamide; \( r = 0.97, p < 0.01 \) for N-acetylprocainamide, no bias in mean results, and random differences of individual determinations. Fifty additional patients' samples containing quinidine from 0 to 12.2 mg/L were quantitated by the current method and by spectrophotofluorometry \( (f) \). The mean concentration by the latter method was 2.8 (SD 2.1) mg/L, and by our method 2.7 (SD 2.4) mg/L \( (r = 0.96, p < 0.01 \).

**Interference by other compounds:** There was no interference with this assay technique by therapeutic concentrations of digoxin (2 mg/L), lidocaine (5 mg/L), phenobarbital (20 mg/L), phenytoin (20 mg/L), propranolol (50 g/L), or theophylline (20 mg/L), nor in blood from patients receiving α-methyldopa (500 mg four times daily), cimetidine (300 mg/d), diazepam (5 mg four times daily), or furosemide (80 mg twice daily).

**Discussion**

The only interference we detected is that of procainamide quantitation in the presence of N-acetylprocainamide. There appears to be a false increase in procainamide of somewhat less than 0.5 mg/L in the presence of 10 mg of N-acetylprocainamide per liter, which probably represents chemical breakdown during the assay procedure. From our experience this should not be clinically significant in the mid-therapeutic procainamide range: these patients usually have N-acetylprocainamide in the 3 to 5 mg/L range, where the interference would average less than 0.25 mg/L. However, in patients who are rapid acetylators, at high drug concentrations, and especially in patients with renal failure, who tend to accumulate N-acetylprocainamide \( (6) \), this crossover may be more significant.

As previously reported \( (15, 16) \) there was significant interference in procainamide quantitation by the early peak seen in blood collected in Vacutainer Tubes containing tris(2-butoxyethyl)phosphate. Blood collected in the older red-top tubes also have abnormalities in quinidine binding and quinidine quantitation because of displacement of the drug by the plasticizer \( (14, 17) \). Furthermore, the two peaks may so perfectly merge as to escape notice, especially at lower sensitivity.

Use of the newer nitrogen–phosphorus detector to quantify tate antiarrhythmic agents gave few problems, once we set up and used this method regularly. Early troubleshooting required the careful selection of voltages and attention to changing the collector to assure reproducible results.

We find this method clinically useful, having quantified both total and unbound drug concentrations in several hundred patients' samples. Procainamide and N-acetylprocainamide concentrations are quite similar to those obtained in our routine hospital laboratories by the EMIT system.

**References**

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Homogeneous Nonisotopic Assay for Phenytoin Evaluated

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We evaluated a new homogeneous immunoprecipitation assay for phenytoin in human serum. No sample dilution or pretreatment is required. The new method is based on spectrophotometry of the inhibition by free phenytoin of the precipitating reaction between anti-phenytoin antibody and a phenytoin-human serum albumin conjugate. A serum test sample is simultaneously mixed with the phenytoin-albumin conjugate and rabbit antiserum to phenytoin in a centrifugal analyzer, and the subsequent reaction is monitored at 3 min. Within-run and between-run coefficients of variation were well below 7%. The relation between results for patients’ test samples as determined by immunoprecipitation assay (y) and an enzyme immunoassay (x) can be expressed as \( y = 1.10x + 1.1 \) (r = 0.966, n = 66).

Additional Keyphrases: centrifugal analyzer • drug assay • immunoprecipitation assay

Several nonisotopic immunoassay methods for determining phenytoin have been reported (1–4) since the inception of the radioimmunoassay method for this drug (5, 6). A recent nonisotopic, homogeneous immunoassay of phenytoin (7) is based on an immunoprecipitation assay (IPA) and avoids the use of enzyme activity, fluorescent tags, radioactive isotopes, or an expensive laser nephelometer. The IPA for phenytoin relates a two-point measurement of absorbance at 340 nm to the concentration of phenytoin in a test sample. Reaction between anti-phenytoin antibody and phenytoin-human serum albumin conjugate increases the absorbance at that wavelength relative to the absorbance of a test blank; reaction of free phenytoin with anti-phenytoin does not. The presence of free phenytoin in a test sample displaces the phenytoin-albumin conjugate from reaction with anti-phenytoin antibody, thus inhibiting the increase of absorbance as compared with that produced in the absence of free phenytoin.

We compared results obtained by the present method with those obtained by an enzyme immunoassay.

Materials and Methods

We used a centrifugal analyzer that incorporates a spectrophotometer (Gemeni; Electro-Nucleonics, Inc., Fairfield, NJ 07006).

Reagents. Anti-phenytoin antibody and phenytoin–albumin conjugate are supplied as lyophilized powders. Accelerator reagent, containing polylethylene glycol (Mw = 6000), is used to reconstitute working antibody reagents. Standard solutions provided contain 0.0, 5.0, 10.0, 15.0, 20.0, and 30.0 mg of phenytoin per liter. All these reagents were provided to us by Electro-Nucleonics Laboratories, Inc., Bethesda, MD 20814.

Procedures. The Gemeni centrifugal analyzer is provided with special polypropylene discs; each disc has 20 cuvettes and each cuvette has two wells, a sample well and a reagent well. The pipetting sequence is as follows: pipet 500 μL of distilled water into the reagent well of cuvettes 1 and 2, the reference wells; 10 μL of standards (0.0, 5.0, 10.0, 15.0, 20.0, and 30.0...