Gas-Chromatographic Determination of Verapamil and Norverapamil, with a Nitrogen-Selective Detector

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We present a procedure for the determination of verapamil and its metabolite, norverapamil, in serum. The drugs are extracted under basic conditions into n-heptane-2-butanol (96/4 by vol) and then extracted again into 1 mol/L H₂SO₄. The acidic solution is made basic with sodium hydroxide, re-extracted with diethyl ether, and the extract evaporated. The residue is redisolved in ethanol and analyzed by gas chromatography with a nitrogen-selective detector. By use of two internal standards, prazeapam and D-517 (a verapamil analog), concentration and instrument response are linearly related from 50 µg/L to 5 mg/L. Within-run precision (CV) was 3 and 5% for both verapamil and norverapamil at concentrations of 100 and 250 µg/L; between-run precision was 11 and 9% at those respective concentrations. Interference studies indicate that most commonly prescribed basic drugs will not interfere with this procedure.

Additional Keyphrases: drug assay • antiarrhythmic drugs • monitoring therapy • toxicology • calcium blocker

Verapamil (Isoptin; Knoll-Pharmaceutical Co., Whippany, NJ 07981), a newly marketed antiarrhythmic drug, is currently available for intravenous administration. The predominant metabolite of verapamil in serum is norverapamil (Figure 1). Norverapamil possesses 20% of the antiarrhythmic activity of verapamil, and its steady-state concentration in serum is equal to that of the parent drug. For optimum therapeutic effect, verapamil concentrations of 100 to 400 µg/L should be attained (1).

Several chromatographic procedures for determining verapamil have been reported (2–5). More recently, a “high-performance” liquid-chromatographic procedure and a gas-chromatographic procedure for determining both verapamil and norverapamil have been reported (1, 6).

We describe a sensitive, rapid, and precise procedure for the routine clinical laboratory determination of verapamil and norverapamil in serum. Optimized gas-chromatographic conditions, extracting solvents, drug interferences, and the use of two internal standards are reported. This procedure can be used for therapeutic monitoring, toxicological workup of patients, and pharmacokinetic studies.

Materials and Methods

Apparatus. We used a Model 5880A gas chromatograph equipped with flame ionization and a nitrogen-phosphorus-selective detector (Hewlett-Packard Co., Avondale, PA 19311). The 1.2 m x 2 mm (i.d.) coiled glass column was packed with 3% SP-2250 Chromosorb W-HP 100/120 mesh (Anspec Co., Ann Arbor, MI 48107). The carrier gas was helium, at a flow rate of 40 mL/min. Injection temperature was 290 °C, oven temperature 290 °C, and detector temperature 300 °C. The nitrogen detector was set at a recorder offset of 70 to 80 mm. Flow rates of hydrogen and air to the glass bead were 3 and 50 mL/min, respectively.

Reagents and standards. All reagents were analytical reagent (AR) grade or “spectral” grade: n-heptane, 2-butanol; absolute methanol; absolute reagent, ACS ether (MC&B Manufacturing Chemists, Inc., Cincinnati, OH 45212). Sodium hydroxide, 0.5 mol/L, and sulfuric acid, 1 mol/L, were prepared from concentrated solutions. n-Heptane-2-butanol (96/4 by vol) extraction solution was prepared from the above analytical reagents. Anhydrous sodium sulfate was used to dry the final ether extract.

Verapamil hydrochloride, norverapamil hydrochloride, and D-517 hydrochloride, a verapamil analog having one fewer methylene group, were obtained from Knoll Pharmaceutical Co. Prazepam was obtained from Warner-Lambert Research Institute, Morris Plains, NJ 07950.

Prazepam and D-517 (internal standards). Dissolve 25 mg of prazeapam and 10.8 mg of D-517 hydrochloride in 100 mL of absolute ethanol. Dilute 1 mL of this to 100 mL with water. This working solution contains 2.5 mg of prazeapam and 1.0 mg of D-517 per liter.

Verapamil and norverapamil. Dissolve 10.8 mg each of verapamil hydrochloride and norverapamil hydrochloride into 10 mL of absolute ethanol. Dilute 1 mL of this to 100 mL with water to prepare an aqueous intermediate standard of 10 mg/L, and then dilute further for aqueous working standards of verapamil/norverapamil of 50 to 1000 µg/L. Use these aqueous standards to reconstitute lyophilized normal human serum (Ortho Diagnostics, Raritan, NJ 08869) to volume.

Procedure. The procedure is a modification of our previously published procedure (7):

Place 2 mL of serum (blank, standard, control, or patient’s sample) into a 50-mL centrifuge tube. Add 0.2 mL of working internal standard solution (prazeapam and D-517) and 4 mL of 0.5 mol/L sodium hydroxide, and vortex-mix. Add 15 mL

![Fig. 1. Structures of verapamil and norverapamil](image-url)
of the n-heptane/2-butanol solution and shake with a mechanical shaker for 5 min. Centrifuge at 5000 rpm for 5 min, remove 12 mL of the top layer, and filter through Whatman No. 1 filter paper into another tube. To the filtrate, add 4 mL of 1 mol/L H2SO4, shake for 2 min, and then centrifuge again for 2 min. To 3 mL of the H2SO4, add 2.5 mL of 4.4 mol/L NaOH, vortex-mix, and add 5 mL of diethyl ether. Shake for 2 min, centrifuge for 2 min, and transfer the ether layer to another tube. Add 1 g of anhydrous sodium sulfate to the ether extract, mix, and then filter it through filter paper. Evaporate the ether and dissolve the residue in 50 µL of absolute ethanol. Inject 3 µL for analysis. Calculate ratios between the peak heights of verapamil and norverapamil and those of the internal standards, prazepam or D-517, then calculate the concentration of the unknowns by comparison with the standard having the most similar peak-height ratio.

Results

Chromatograms of a serum blank, standards, and a patient’s sample are shown in Figure 2. Blank sera assayed by this procedure had no peaks that interfered with the analysis. Retention times for prazepam, verapamil, and norverapamil are 0.40, 1.26, and 1.56 relative to D-517, which has a retention time of 3.12 min.

When D-517 was used for calculating peak-height ratios, least-squares analysis results from serum extracts containing 50, 100, 250, 500, and 1000 µg of verapamil per liter gave a slope of 0.0072, a y-intercept of −0.0112, a standard error of estimate Sxy of 0.119, and a correlation coefficient (r) of 0.999. For norverapamil, the slope was 0.0036, the y-intercept −0.080, Sxy = 0.082, and r = 0.998. Similar results were obtained when prazepam was the internal standard for the calculations. The linearity of the standard curve extended to 5 mg/L with the use of prazepam as internal standard. Least-squares analysis of results from sera containing 25, 50, 75, and 100 µg each of verapamil and norverapamil per liter and D-517 as internal standard gave slopes of 0.0293 and 0.0169, y-intercepts of −0.23 and −0.186, standard errors of estimate of 0.049 and 0.026, respectively, and correlations of 0.999 for each. For the linearity study from 25 to 100 µg/L, the amount of internal standard used was decreased to one-third of the original value. The sensitivity of the method (twice the noise or background) is 5 µg/L for verapamil and 10 µg/L for norverapamil.

The absolute recovery of drugs added to sera in the 50 to 1000 µg/L range (five concentrations) averaged 84 (SD 7)% for verapamil and 46 (SD 7)% for norverapamil. The relative percent recovery of serum standards (n = 5) in the same concentration range (50–1000 µg/L) averaged 100 (SD 9)% each for verapamil and norverapamil.

Within-run precision (CV) of the assay at 100 and 250 µg/L concentrations (n = 5 each) was 2 and 3% for verapamil and 5% for norverapamil. Precision of the assay at 10 µg of verapamil and norverapamil per liter was 6 and 8%, respectively (n = 5 each). Between-run CV (n = 10) at 100 and 250 µg/L was 11 and 7% for verapamil and 11 and 10% for norverapamil.

Interference studies indicate that midazolam interferes with prazepam, being extracted under the conditions of the present method and having the same retention time as prazepam. The following drugs, which all can be extracted from a basic solution, will not interfere: propanamide, quinidine, diisopyramide, lidocaine, propranolol, propoxyphene, sulfanilamide, loxapine, imipramine, desipramine, doxepin, desmethyldoxepin, amitriptyline, nortriptyline, oxazepam, diazepam, nordiazepam, flurazepam, desalkylflurazepam, chloridiazepoxide, desalkylchloridiazepoxide, amphetamine, antihistamines, methaqualone, and protriptyline. No interferences were observed from such more-acidic drugs as glutethimide, barbiturates, meprobamate, phenytoin, ethchlorvynol, acetaminophen, and salicylate.

Discussion

Verapamil, the prototype calcium blocker, is currently available for intravenous administration. Its β-phase half-life reportedly varies from 3 to 7 h in normal persons given intravenous and oral doses (2, 8–11). Differences in the half-life of the drug have been reported in liver-disease patients and in intensive-care patients (9). The bioavailability of the drug given orally is low (only 20%), owing to hepatic first-pass metabolism (11). Because of these large observed variations in half-life and low bioavailability, concentrations of the drug should be measured in serum to determine proper dosage and optimum therapeutic efficacy (1, 9). Verapamil is extensively metabolized in humans, the major active metabolite being
norverapamil (1,12). Although therapeutic ranges have not been established for norverapamil, the measurement of both verapamil and norverapamil in serum is a better guide for optimizing therapy.

Using the present procedure, we observed excellent linearity of instrument response for verapamil and norverapamil in the 25–1000 μg/L range. Within-run CV averaged 5%, between-run 10%. The use of two internal standards, prazepam and D-517, is necessary to expand the range of linearity from 50 μg/L to 5 mg/L. Thus this procedure can be used to analyze samples from patients on long-range verapamil therapy, to evaluate intravenous administration, and for pharmacokinetic and toxicological studies. A case involving a fatal overdose of verapamil (concentration in blood, 8.8 mg/L) has been reported (13). Our procedure can be used for such an analysis because its response is linear with concentration to at least 5 mg/L. The applicability of this procedure in following cases of patients treated with verapamil is illustrated in Table 1. Patient B.R. was started on intravenous verapamil and was then switched to an oral dose. Verapamil concentrations ranged from 304 to less than 10 μg/L. Norverapamil was observed in the patient’s sample 24 h after the patient was switched over to the oral dosage. Three weeks later the patient had attained respective steady-state concentrations of 225 and 159 μg/L for verapamil and norverapamil. Notice the large variability of concentration of parent drug and metabolite in patients on similar dosages of verapamil.

Recovery for the metabolite norverapamil (46%) is low, probably because it is a secondary amine and therefore is not as readily extracted as the parent compound, which is a tertiary amine. [Similar results have been reported for disopyramide and its metabolite, desalkyldisopyramide (14).] Thus the use of two internal standards is important in analyzing a parent drug and a metabolite with different extraction properties. For equal concentrations of verapamil and norverapamil, one would need twice the concentration of norverapamil to obtain an equivalent detector response.

Verapamil has two other metabolites, D-620 and D-617, which are formed by dealkylation at the nitrogen group; their retention times are <1 min under these chromatographic conditions. Although these metabolites are extracted by this procedure, they are not separated on a 3% SP-2250 column and cannot, therefore, be determined by gas chromatography. With “high performance” liquid chromatography and a C-18 column, verapamil, norverapamil, D-617, D-620, and prazepam are readily separated, with retention times of 0.70, 0.51, 0.40, and 0.33 relative to prazepam (16 min). The mobile phase was methanol/water (60/40 by vol) at pH 3.5 with octanesulfonic acid added for ion pairing (unpublished results).

Of the many gas-chromatographic procedures reported, only one has been used for quantitative determination of verapamil and norverapamil in serum (1); these drugs were measured in patients on chronic maintenance therapy. The only internal standard reported (1, 3, 4) was the verapamil analog, D-517, and one method (2) had no internal standard. More recently, a liquid-chromatographic procedure for verapamil and norverapamil in serum has been reported in which D-517 is the internal standard (6). Responses of the previous gas- and liquid-chromatographic procedures were linear with concentration up to 500 μg/L; by using two internal standards our linearity range is from 50 μg/L to 5 mg/L, which eliminates the need of re-extracting samples and re-analyzing them when they fall outside of the more limited linearity range. Besides improving the quantitation of parent drug and metabolite, the use of two internal standards also serves to eliminate interferences. Verapamil and norverapamil are readily and precisely separated on a 3% SP-2250 column at 290 °C and analysis time is less than 6 min per sample. In contrast, the analysis times by liquid chromatography exceed 15 min.

Table 1. Concentrations of Verapamil and Norverapamil in Sera of Patients Treated with Verapamil

<table>
<thead>
<tr>
<th>Patient</th>
<th>Time of sampling</th>
<th>Concentration, μg/L</th>
<th>Verapamil</th>
<th>Norverapamil</th>
</tr>
</thead>
<tbody>
<tr>
<td>B.R.</td>
<td>4/28, blank (pre drug)</td>
<td>0.0</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4/28, 0 min (end of infusion)</td>
<td>304</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4/28, 6 min</td>
<td>140</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4/28, 12 min</td>
<td>116</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4/28, 30 min</td>
<td>72</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4/28, 2 h</td>
<td>64</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4/28, 12 h</td>
<td>15</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4/29, 17 h (after end of infusion)</td>
<td>&lt;10</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5/01, 48 h (after switched to oral dosage)</td>
<td>47</td>
<td>40.0</td>
<td></td>
</tr>
<tr>
<td>M.R.</td>
<td>5/22, 80 mg orally</td>
<td>225</td>
<td>159.0</td>
<td></td>
</tr>
<tr>
<td>T.H.</td>
<td>8/18, 80 mg orally</td>
<td>99</td>
<td>129.0</td>
<td></td>
</tr>
<tr>
<td>V.O.</td>
<td>8/18, 80 mg orally</td>
<td>55</td>
<td>87.0</td>
<td></td>
</tr>
<tr>
<td>M.M.</td>
<td>6/19, 80 mg orally</td>
<td>136</td>
<td>114.0</td>
<td></td>
</tr>
<tr>
<td>L.A.</td>
<td>8/05, 80 mg orally</td>
<td>63</td>
<td>70.0</td>
<td></td>
</tr>
</tbody>
</table>

* B.R. was given verapamil (0.1 mg/kg body wt.) as a bolus on 4/28, followed by an infusion of 7 μg/kg per minute for 20 min. On 4/29, verapamil was given orally 1 mg/kg three times a day. On 4/30, verapamil was changed to 1 mg/kg four times a day.

References


