Acetone and methanol failed to react; however, ethylene glycol (4.00 g/L) and isopropanol (1.50 g/L) produced false-positive reactions in 10 min equivalent to 0.40 g of ethanol per liter.

Discussion

The major advantage of the new ADH-glycine assay over the manufacturer's previous ADH-pyrophosphate assay is the shorter time (10 min) required for the reaction to be completed. Our laboratory has routinely performed ADH ethanol determination for 15 years, and during this period we noted that the ADH-pyrophosphate assay often required 30 min for reaction completion. As the pyrophosphate buffer ages, reaction times occasionally increase to 45 min. While we have only six months' experience with new assay, we have not observed any lengthening of the required reaction time.

The major disadvantage of the new assay is that shared by all ADH assays: reaction with alcohols other than ethanol (3–4). The rate of oxidation of these alcohols decreases in the following order: ethanol = allyl alcohol > n-propanol > n-butanol > n-amyl alcohol > isopropanol (5). In our experience, isopropanol is the only reacting alcohol, other than ethanol, that may occasionally be encountered clinically. Gas-chromatographic analysis of blood, before ADH determination of ethanol, would indicate the presence of isopropanol or other volatile substances (6). Acetone, methanol, sec-butyl, and isobutyl alcohol are not oxidized by ADH.

The new ADH-glycine assay gave precise and accurate measurement of blood ethanol concentrations. The results of the new assay compare well with those obtained by the previous ADH assay and by gas chromatography.

We thank Sigma Chemical Co., St. Louis, MO, for generously supplying the ADH reagents necessary for this study.

References


Simultaneous Liquid-Chromatographic Determination of Carbamazepine and Its Epoxide Metabolite in Plasma

Ronald J. Sawchuk and Linda L. Cartier

We describe a liquid-chromatographic method for the simultaneous quantification of carbamazepine and its 10,11-epoxide metabolite in plasma. The method is used routinely in the analysis of carbamazepine and its epoxide in 0.5 mL of plasma at concentrations of 1 to 20 and 0.2 to 5 mg/L, respectively. The use of peak-height ratios as a measure of detector response appeared to provide better precision and accuracy than peak-area ratios.

Additional Keyphrases: drug assay • anticonvulsants • epilepsy • standard-addition method • therapeutic concentrations

Carbamazepine (CBZ), an effective anticonvulsant commonly used in the treatment of grand mal and psychomotor epilepsy, is also the drug of choice in trigeminal neuralgia. In studies demonstrating a relationship between serum or plasma concentration of carbamazepine and degree of seizure control (1–5), in general the therapeutic range appears to be 4 to 10 mg/L. The 10,11-epoxide metabolite (CBZE), however, also appears to be an active anticonvulsant (6), and variation in therapeutic response to a given concentration of CBZ in serum may be explained in part by the presence of steady-state concentrations of the epoxide metabolite (7).

Several methods of analysis for CBZ in plasma have been described: spectrophotometry, fluorometry, gas–liquid chromatography, “high-pressure” liquid chromatography (8–10). Methods have also been reported for the simultaneous measurement of CBZ and CBZE in human plasma. A previously reported (11) liquid-chromatographic method for CBZ and CBZE appears to be adequately sensitive for CBZ but, because of interference from an endogenous compound that co-elutes with CBZE, its sensitivity for the metabolite is somewhat limited. Analysis for CBZ and CBZE by a method involving precipitation of plasma proteins is not suitable because phenytoin, an anticonvulsant that is commonly used with CBZ, interferes (12).

The observation that CBZE may contribute to the anticonvulsant effect of the parent drug suggests that monitoring both CBZ and CBZE may be of value. In addition, if the induction of CBZ caused by its prior administration occurs via the epoxide pathway, the CBZE/CBZ ratio during chronic

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dosing should provide an index of this phenomenon. We describe a rapid and precise method for quantifying CBZ and CBZE in plasma in concentrations as low as 20 μg/L.

**Materials and Methods**

**Instrumentation.** For liquid chromatography we used a “high-pressure” liquid chromatograph (Model 1084B; Hewlett-Packard, Palo Alto, CA 94304) equipped with a variable (190–660 nm) wavelength detector and an automatic sampling system. The separation is carried out on a 15 cm × 4.6 mm (i.d.) prepacked, microparticulate (5-μm av particle size), reversed-phase column (LC-18; Supelco, Inc., Bellefonte, PA 16823). The flow rate of the mobile phase, methanol/distilled water (45/55 by vol), is 2.5 mL/min. The column effluent is monitored at 212 nm, at attenuations ranging from 2 to 27, depending on the concentration of the samples (or standards) being analyzed. Chart speed is ordinarily 0.5 cm/min.

**Reagents.** Chemicals used were carbamazepine and carbamazepine-10,11-epoxide (Ciba-Geigy Corp., Ardsley, NY 10502); cytebatidine, 1 g/L in methanol (Supelco, Inc.); chloroform, distilled in glass, without ethanol preservative (Burdeck and Jackson Labs., Inc., Muskegon, MI 49442); methanol (Omnisolv; Matheson, Coleman and Bell Manufacturing Chemists, Inc., Cincinnati, OH 45212); potassium phosphate monobasic (AR grade; Mallinckrodt, Inc., St. Louis, MO 63160); and sodium hydroxide (reagent grade; Matheson, Coleman and Bell).

**Sample extraction and chromatography.** To a series of six 35-mL ground-glass-stoppered reaction vessels add CBZ in methanol (0.05 g/L) in amounts of 0.5, 1.25, 2.5, 5.0, and 10.0 μg to prepare the standard curve. Likewise, add CBZE in methanol (0.01 g/L) in amounts of 0.1, 0.25, 0.5, 1.0, and 2.5 μg to the same tubes. Add 3.75 μg of cytebatidine in methanol (0.25 g/L) to these tubes and remove the methanol under reduced pressure. Add an identical amount of cytebatidine to a series of clean 35-mL tubes for the samples, and allow the methanol to evaporate. Add 0.5 mL of blank plasma to each tube in the standard-curve series, and 0.5 mL of plasma to be analyzed to the sample tubes. Add 1.0 mL of phosphate buffer (0.1 mol/L, pH 7.4). Add 10 mL of chloroform, stopper, and shake horizontally at 180 cycles per minute on a mechanical shaker (Eberbach Corp., Ann Arbor, MI 48106) for 5 min. Centrifuge for 5 min at 750 × g. Aspirate and discard the aqueous phase. Transfer the chloroform phase to a clean 15-mL glass-stoppered centrifuge tube, and evaporate the solvent on an evaporator (Evapo-Mix; Buchler Instruments, Fort Lee, NJ 07024).

Dissolve the residue in 200 μL of mobile phase by vortex-mixing, and transfer to a microvial for automatic injection. Inject 10 μL of the sample, using the chromatographic conditions described above. Measure peak heights manually and calculate the ratio of peak heights of CBZ and CBZE to those of the internal standard CHA.

**Calculations.** Standard curves constructed for peak height ratios are adjusted by linear regression analysis to express peak-height ratio as a function of CBZ and CBZE concentration of the standards. The CBZ and CBZE concentrations in the samples are calculated from these equations.

**Results and Discussion**

**Assay Characteristics**

**Linearity and precision.** Standard curves for CBZ and CBZE in the range of 1 to 20 and 0.2 to 5.0 mg/L, respectively, exhibited good linearity (Table 1 and Figure 1). Correlation coefficients and slopes for these relationships were determined from peak-height ratio for both drug and metabolite as indi-

### Table 1. Analytical Precision of the Analysis for Carbamazepine and Carbamazepine-10,11-epoxide (Peak Height Ratios Used)

<table>
<thead>
<tr>
<th>Carbamazepine assay precision (Peak Height Ratios Used)</th>
<th>Carbamazepine-10,11-epoxide assay precision (Peak Height Ratios Used)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Within-day</strong> a</td>
<td><strong>Between-day</strong> b</td>
</tr>
<tr>
<td><strong>Conc., mg/L</strong></td>
<td><strong>Peak height, ratio, mean ± SD</strong></td>
</tr>
<tr>
<td>0.0</td>
<td>0.000</td>
</tr>
<tr>
<td>1.0</td>
<td>0.269 ± 0.001</td>
</tr>
<tr>
<td>2.5</td>
<td>0.690 ± 0.011</td>
</tr>
<tr>
<td>5.0</td>
<td>1.48 ± 0.095</td>
</tr>
<tr>
<td>10.0</td>
<td>2.78 ± 0.014</td>
</tr>
<tr>
<td>20.0</td>
<td>5.93 ± 0.067</td>
</tr>
<tr>
<td>Slope</td>
<td>0.2964 ± 0.0035</td>
</tr>
</tbody>
</table>

a Three sets of standard curves analyzed on the same day. b Three sets of standard curves analyzed on three different days. c CV of the slopes.
Table 2. Results of Simultaneous Analysis for Carbamazepine and Carbamazepine-10,11-epoxide in Five Human Plasma Samples on Different Days

<table>
<thead>
<tr>
<th>Added concn, mg/L</th>
<th>Average (±SD) measured concn, mg/L</th>
<th>Average (±SD) recovery, %</th>
<th>Added concn, mg/L</th>
<th>Average (±SD) measured concn, mg/L</th>
<th>Average (±SD) recovery, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbamazepine</td>
<td></td>
<td></td>
<td>Carbamazepine-10, 11-epoxide</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.0</td>
<td>1.03 ± 0.087</td>
<td>103 ± 8.7</td>
<td>0.20</td>
<td>0.194 ± 0.0064</td>
<td>97 ± 3.2</td>
</tr>
<tr>
<td>5.0</td>
<td>4.97 ± 0.088</td>
<td>99 ± 1.6</td>
<td>0.50</td>
<td>0.496 ± 0.031</td>
<td>99 ± 6.2</td>
</tr>
<tr>
<td>10.0</td>
<td>10.5 ± 0.64</td>
<td>105 ± 6.4</td>
<td>1.0</td>
<td>1.11 ± 0.094</td>
<td>111 ± 9.4</td>
</tr>
<tr>
<td>20.0</td>
<td>21.4 ± 1.6</td>
<td>107 ± 7.9</td>
<td>5.0</td>
<td>5.6 ± 0.48</td>
<td>113 ± 9.8</td>
</tr>
</tbody>
</table>

Spectra were consecutively accumulated for five replicate analyses of each plasma sample containing CBZ and CBZE at each of four concentrations. These were studied to determine the degree of interference at each concentration level.

Analytical recovery. Known amounts of CBZ and CBZE were added to plasma in concentrations ranging from 1.2 to 19.5 mg/L (CBZ) and 0.2 to 4.9 mg/L (CBZE). The samples (n = 38) were prepared by an independent group and shipped to our laboratory for analysis as part of a blind study to validate the analysis. Mean analytical recovery, expressed as the ratio of compound added to that measured, was 95% (SD 4.9%) for CBZ and 95% (SD 9.9%) for CBZE. Recovery was further calculated for five replicate analyses of each plasma sample containing CBZ and CBZE at each of four concentrations. Two sets of analysis done on different days are summarized in Table 2.

Physical recovery. The physical recovery of CBZ and CBZE was determined by comparing the peak-height ratios measured in the extracts of plasma containing known amounts of both compounds with those peak-height ratios measured in unextracted samples supplemented with known amounts of CBZ and CBZE. For purposes of this calculation, we added the internal standard to the samples just before injection into the chromatograph. Recoveries of CBZ and CBZE determined in this manner were found to be 85% and 78%, respectively.

Sensitivity. Using a signal/noise ratio of 2/1, the minimum detectable quantity on column is 0.8 ng for both CBZ and CBZE. By injecting 180 µL of the 200-µL reconstituted plasma extract, one can detect drug and metabolite in concentrations as low as 1.1 µg/L. Under these conditions, both of these compounds can be quantified with acceptable precision at concentrations of 20 µg/L in 0.5 mL of plasma.

Specificity. Figure 2 shows typical chromatograms obtained in the analysis of blank and drug-supplemented human plasma. No interfering peaks are noted in blank plasma. Spectra of CBZ and the epoxide metabolite were obtained in stop-flow mode during chromatography of the compounds, both extracted from plasma and unextracted. In each case (CBZ and CBZE) the spectra from unextracted and extracted samples were qualitatively similar.

When the chromatographic behavior of three other commonly prescribed anticonvulsants was examined under our chromatographic conditions, none of these was found to interfere with CBZ or CBZE. Their retention times relative to that of CBZ were: primidone, 0.25; phenobarbital, 0.37; CBZE, 0.45; and phenytoin, 0.85.

Although there is no significant difference between values calculated from peak-area ratio and peak-height ratio response for CBZ, measurements of peak heights provide greater accuracy in the determination of concentrations of CBZE in plasma. Similarly, run-to-run precision is improved in CBZE analysis by using peak-height measurements. We therefore recommend use of peak-height ratios over peak-area ratios for CBZ and CBZE plasma analysis with this procedure.

![Fig. 2. Representative chromatograms showing retention times of (1) CBZE, (2) CBZ, and (3) cyheptamide (internal standard) in 0.5 mL of plasma](image)

A, patient's plasma containing CBZE (0.23 mg/L) and CBZ (1.16 mg/L); B, patient's plasma containing CBZE (4.67 mg/L) and CBZ (18.3 mg/L); C, blank plasma supplemented with internal standard. Mobile phase: methanol/water, 45/55 by vol; flow rate, 2.5 mL/min; wavelength 212 nm.
use of time-programmed attenuation changes allows one to select an optimum sensitivity so that the height of each peak can be measured with acceptable precision.

Measured Concentrations of Carbamazepine and Its Epoxide in Plasma from Epileptic Children at Steady State

The present method was used to characterize the bioavailability of a suspension of carbamazepine in epileptic children. The study was carried out under steady-state conditions. We determined concentrations of CBZ and CBZE in the plasma of 18 children who were receiving the suspension in therapeutic dosages over one dosing interval. Average concentrations for CBZ were 8.1 (range, 4.1–11.2) mg/L and for CBZE 1.6 (range 0.91–3.5) mg/L. Although the average of the CBZE/CBZ plasma concentration ratio (0.20) was similar to that reported by others, this ratio was not as variable as that previously reported in children (13).

The sensitivity of the present method is adequate for the analysis for CBZ and CBZE in plasma of subjects receiving single doses of CBZ. The linearity and precision of the method also permit analysis of both drug and metabolite over a wide range of concentrations in plasma.

We thank Dr. Keith Chan, Research and Development Department, Pharmaceuticals Division, Ciba-Geigy Corp., Ardsley, NY 10502, for supplying pure carbamazepine and carbamazepine-10,11-epoxide, and for preparing the plasma samples for the blind study to validate the analysis.

References


