Propylene Glycol Interference in Gas-Chromatographic Assay of Ethylene Glycol

To the Editor:

We wish to substantiate the important observations of LeGatt and Tisdell (1) cautioning against the use of propylene glycol as an internal standard for quantifying ethylene glycol. A 32-year-old woman presented to our emergency department in an agitated state after the ingestion of multiple drugs in an attempted suicide. Gastric lavage and charcoal were administered as well as intravenous diazepam (Valium) and lorazepam (Ativan) for sedation. While attempting to quantify ethylene glycol in her serum by gas chromatography, we observed that the specimen contained a compound that was eluted with a retention time similar to that of propylene glycol, the internal standard. Although ethylene glycol was undetectable, concentrations of propylene glycol at admission as well as in subsequent serum specimens obtained over the next 35 h were substantial (Table 1). Propylene glycol concentrations peaked 10–15 h after admission to the hospital. Subsequently it was determined that Ativan and Valium contained propylene glycol, 80% and 40%, by weight, respectively. Calculations based on the administered doses of these two benzodiazepines revealed that ~18 g of propylene glycol had been given. In addition, lactic acidosis (Table 1) was observed, presumably as a consequence of propylene glycol toxicity (2). Thus, as previously described in another patient for whom measurement of ethylene glycol was requested, we found propylene glycol in serum after treatment with intravenous formulations that contained propylene glycol. Therefore, in measurements of ethylene glycol by gas chromatography, 1,3-propanediol (e.g.) should be used as an internal standard (1) to avoid interference from propylene glycol.

References

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Table 1. Propylene Glycol Concentrations in Serum after Administration of Large Doses of Benzodiazepines

<table>
<thead>
<tr>
<th>Time after admission, h</th>
<th>Propylene glycol, mg/L (mmol/L)</th>
<th>Lactate, mmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>210 (2.76)</td>
<td>—</td>
</tr>
<tr>
<td>5</td>
<td>480 (6.32)</td>
<td>—</td>
</tr>
<tr>
<td>10</td>
<td>1220 (16.05)</td>
<td>—</td>
</tr>
<tr>
<td>15</td>
<td>1240 (16.32)</td>
<td>5.2</td>
</tr>
<tr>
<td>20</td>
<td>690 (8.95)</td>
<td>2.7</td>
</tr>
<tr>
<td>30</td>
<td>160 (2.11)</td>
<td>1.6</td>
</tr>
</tbody>
</table>

DagaBita et al. (2) recently examined the analytical performance of another immunoassay allegedly specific for parent CsA, the EMIT™ cyclosporine assay (Syva Co., Palo Alto, CA). These investigators also measured CsA concentrations in 196 whole-blood samples from transplant patients by this assay, by HPLC, and by RIA (InoStar Cyclo-Trac SP). They reported "a small positive bias between the EMIT assay (y) and HPLC (x) as reflected by the slope of 1.27" (y-intercept = 16.4), and emphasized the good correlation (r = 0.96) between the methods (the standard error of the estimate, S(y|x), was 34.9 µg/L). Actually, the degree of agreement between these methods was poor. For example, for a CsA value of 100 µg/L by HPLC, the EMIT method would on the average give a result of 143 µg/L, and we are 95% sure that the value would be between 73 and 213 µg/L (3).

Correlation coefficients have been widely misused as an indicator of between-method agreement in studies comparing different methods for CsA measurement. This statistic does no such thing. A correlation coefficient measures the strength of the relation between the results by two methods, not the agreement between them. A set of measurements that cluster closely around any straight line, not necessarily around the line of identity, will result in a high correlation coefficient (4).

In addition to the slope and y-intercept, the standard error of the estimate is essential information in the interpretation of linear-regression analysis. The standard deviations of the slope and y-intercept should also be reported. These issues were discussed in the recent Canadian consensus meeting on CsA monitoring (5). Moreover, spurious data may pose a major source of error in linear-regression analysis; this may have been the case in the study of Moyer et al. (1). It is therefore important always to plot the data.

A properly performed linear-regression analysis can yield a great deal of useful information about how well two methods compare. The microcomputer revolution has made it easy to perform regression analysis of large quantities of measured data, and some caution is needed to avoid pitfalls in the interpretation of the results.

References

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Editor's Note: The above recommendations mirror those in our Information for Authors. We think they are worth repeating, in view of the high frequency with which we ask authors to observe them.

Monitoring Cyclosporine by HPLC with Cyclosporin C as Internal Standard

To the Editor:

Cyclosporin A (CsA, cyclosporine) is currently the immunosuppressant drug of choice for organ-transplant patients. Monitoring the concentration of CsA is often critical to patient management because of the adverse side effects and extremely individual metabolic variability of this drug. HPLC has long been considered the "gold standard" methodology for monitoring CsA concentrations. Cyclosporin D (CsD), the form of cyclosporine traditionally used as the internal standard for HPLC assays, has become unavailable since Sandoz stopped supplying it (personal communication, Kathleen Roskaz, Inventory Control, Sandoz Research Institute, East Hanover, NJ) and as stocks have run out. Here we show that cyclosporin C (CsC) can be used as an alternative internal standard with some modifications of the original procedure of Moyer et al. (1).

The HPLC we used to determine CsA was a Perkin-Elmer (Norwalk, CT) system with a Supelco (Bellefonte, PA) 5-cm LC-1 column and ultraviolet detection at 214 nm. This is an isocratic, reversed-phase system. The modifications made to the original procedure were: (a) changing the mobile phase from acetonitrile/methanol/water (3:3:4 by vol) to acetonitrile/water (46:54 by vol); (b) slowing the flow rate from 1.4 to 1.2 mL/min; (c) extending the run time from 11 to 14 min; and (d) substituting CsC for CsD.

After modifying the procedure to use CsC as the internal standard, we ran precision and linearity studies, as well as a correlation study with patients' samples. Between- and within-run precisions were determined at two concentrations of CsA-supplemented whole-blood controls. Between-run (n = 14) mean CsA concentrations were 174.3 and 512.4 μg/L, and the corresponding CVs were 4.7% and 4.0%. For the within-run (n = 9) precision study, mean CsA concentrations of 169.6 and 496.1 μg/L yielded CVs of 5.1% and 4.9%, respectively.

To determine the linearity and sensitivity of the revised procedure, we prepared blank whole-blood samples, then added CsA at concentrations ranging from 12.5 to 2000 μg/L (the range of linearity of the method of Moyer et al.). We found that the results of the modified method varied linearly with concentration in the range of 25–1000 μg/L, with a correlation between theoretical and actual values (r) of 1.00 and a regression equation of y = 1.00x – 4.37 μg/L. Unlike the original method, the modified method did not detect the 12.5 μg/L sample.

To study the correlation between the two methods, we assayed a series of samples from pediatric liver (n = 45), kidney (n = 29) transplant patients and adult heart (n = 55) transplant patients. Any samples arriving in the laboratory for routine CsA determination that exceeded 4 mL of EDTA-treated whole blood were assayed by both methods. Figure 1 shows the regression and bias plots of results from those samples with CsA concentrations <400 μg/L. We present these data (n = 119) instead of those for all of the patients' samples (n = 129) to more clearly demonstrate the arrangement of data in the concentration range that includes most of the CsA values. The overall correlation for patients' samples was y = 0.989x + 1.66 μg/L (r = 0.981); for the 0–400 μg/L data shown in Figure 1, r = 0.982. As shown, the CsC method demonstrates neither a negative nor a positive bias in comparison with the original method. The scatter in the bias plots appears to be about equal on both sides of the line.

When we considered the data from each organ type separately, the regression equation for the liver-transplant patients was y = 1.064x – 14.5 μg/L (r = 0.989), for the renal patients was y = 0.948x + 6.39 μg/L (r = 0.989), and for the heart patients was y = 0.860x + 21.8 μg/L (r = 0.954).

Fig. 1. Regression (left) and bias (right) plots for patients' samples with CsA values <400 μg/L, used for comparison of CsA values determined by HPLC with CsD vs CsC as internal standard

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