Table 2. \(p_{aH}\) Values (Determined without Liquid Junction) Compared for Bic, Bicine, and Phosphate Buffers in Isotonic Saline Media, and Pipes Buffer in Water

<table>
<thead>
<tr>
<th></th>
<th>(m_1/\text{mmol} \cdot \text{kg}^{-1}) (acid)</th>
<th>(m_2/\text{mmol} \cdot \text{kg}^{-1}) (salt form)</th>
<th>(m_3/\text{mmol} \cdot \text{kg}^{-1}) (NaCl)</th>
<th>(t. , ^\circ\text{C})</th>
<th>Bic</th>
<th>Bicine</th>
<th>Pipes</th>
<th>(H_2PO_4^-/HPO_4^{2-})</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>6.934</td>
<td>6.876</td>
<td>5.340</td>
<td>7.297</td>
</tr>
</tbody>
</table>

*Ref. 7.*

and sodium Pipesate buffers should be satisfactory for use in control of pH near neutrality, in biomedical research, clinical chemistry, acid-base equilibria studies, and other investigations involving chemical kinetics.

The \(p_{aH}\) values clearly indicate that the effect of reduction in concentration (or the dilution effect) on \(p_{aH}\) is small for these buffers. For example, at 37\(^\circ\text{C}\), \(p_{aH}\) was 6.732 for 20 mmol kg\(^{-1}\) equimolar Pipes buffer, whereas at the same temperature, it is 6.575 for 100 mmol kg\(^{-1}\) equimolar buffer. In addition, \(dp_{aH}/dt\) (the temperature coefficient) is equal to approximately \(-0.0062 \, \text{pH/}^\circ\text{C}\) near room or body temperature, which closely resembles that of plasma (\(-0.010 \, \text{pH/}^\circ\text{C}\) and whole blood (\(-0.015 \, \text{pH/}^\circ\text{C}\)). Both of these phenomena increase the usefulness of this proposed buffer.

We expect that more exhaustive treatment of the data concerning a large number of buffers will soon be published in Crit. Rev. Anal. Chem. in the form of a jointly-authored review article from this laboratory and that of R. G. Bates (University of Florida). It will compare the \(p_{aH}\) values (without liquid junction) for several different buffers (6) in water and in isotonic saline, with the pH(s) values (with liquid junction). Compounds whose buffer properties are currently under investigation include glycine derivatives such as N-[tris(hydroxymethyl)methyl]glycine ("Tricine") and N,N-bis(2-hydroxyethyl)glycine ("Bicine"), taurine derivatives such as 2-[bis(2-hydroxyethyl)amino]ethanesulfonic acid ("Bes") and Pipes, and other structurally unrelated compounds such as (carbamoylmethyl)iminodiacetic acid ("Ada") and cholamine chloride.

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References


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Enzyme Immunoassay and Gas-Liquid Chromatography Compared for Determination of Valproic Acid in Serum

To the Editor:

Increasingly, physicians have recourse to the therapeutic monitoring of valproic acid (VPA), an effective drug for the treatment of absence seizures, grand mal, and myoclonic epilepsy (1). Hitherto, determinations of VPA in serum have been performed by gas-liquid chromatography (GLC). Recently, a new enzyme immunoassay (EMIT\(^\text{TM}\), Syva Co., Palo Alto, CA) has been introduced. We compared this new method with GLC for the assay of VPA in serum or plasma from treated epileptic patients. We analyzed 78 sera or heparinized plasmas from epileptic patients receiving VPA, by both GLC and EMIT. None of these samples was lipemic, icteric, or severely hemolytic.

GLC was done according to our previously published method (2). Results so obtained for 15 samples provided by an interlaboratory quality-control scheme (3) agreed well with results from the other participant laboratories. The regression of our values vs the means of the accepted values reported by the participants was: our value = 0.992 mean value - 0.2 (mg/L), \(r = 0.988\), standard error of the estimate 6.0 mg/L.

The EMIT technique was applied as described in the Syva EMIT\(^\text{TM}\) package insert (4). The only modification concerns the instrument used. A Perkin-Elmer spectrophotometer set at 450 nm was used in the concentration mode (factor: 2.667) with the autosampler unit, the 8-\(\mu\text{L}\) quartz cuvet heated at 30 ± 0.1 \(^\circ\text{C}\), the system controller with a print delay of 3 s, and the print-timer with a measured time of 15 s. The differences in absorbance at 15 and 45 s after the print delay period were calculated, to determine the standard curve and the concentration of the samples. This instrumentation was fully satisfactory for other EMIT assays.

The data were statistically evaluated by linear least-squares regression analysis (classical and Deming's methods) (5). The results were interpreted according to Westgard and Hunt (6).

Our results are as follows.

The concentrations in the 78 patients' samples as determined by GLC ranged from 0 to 129 mg/L. By EMIT, four samples gave values <10 mg/L (GLC values: 0), five samples were found to be >150 mg/L (GLC values: 116 to 129), three samples gave irreproducible absorbance differences, and one sample was considered to be an outlier (GLC: 47, EMIT: 94). These 13 samples were omitted from the calculations.

Results of the statistical analysis performed by Deming's method on the concentrations (mg/L) of the 65 remaining samples are: \(r = 0.956\); regression equation EMIT (y) vs GLC (x): \(y = 1.258x - 0.79\). For example, a concentration of 60 mg/L as determined by GLC would correspond to one of 74.7 mg/L by EMIT. The difference between the slope value and unity has a t value of 5.879 \((p < 0.001)\). Thus we found a highly significant proportional error between the two methods.

Six control samples were also determined by the two methods. Four of these samples were purchased from commercial companies and were prepared in our laboratory by adding known amounts of VPA to pooled human drug-free serum (2). The results...
EMIT calibrators thus yield results equivalent to their expected (supplemented) values.

We estimated within-run precision of the EMIT assay by duplicate analysis of the 65 patients' samples. The CV was 9.4%. As suggested in the kit protocol, when the duplicate readings differed by more than 0.006 absorbance unit, a third complete assay was performed and the two closer absorbance differences were averaged. With this procedure, the CV decreased to 6.3%. These precision data are consistent with those claimed by Syva (4).

The repeatability of our GLC method is 5.1% (2).

We have thus found a highly significant proportional error of 26% between EMIT and GLC results for patients' specimens. On the other hand, the two methods give equivalent results for control samples and for the EMIT calibrators.

In an EMIT/GLC comparison study for VPA (7) no difference was found, the classical regression being \( EMIT = 0.95 \times GLC - 0.78 \) (mg/L), \( n = 56 \). In the Syva Summary Report (8), five method-comparison studies are reported. The slopes of the regression lines found in these studies range from 0.987 to 1.133, and the intercepts from −0.94 to −5.90 mg/L. No statistical evaluation of these figures is given.

That our EMIT values are higher than our GLC values for patients' samples could be explained by the cross reactivity of some VPA unsaturated metabolites, which reportedly interfere with the enzyme immunoassay (4). Three of these metabolites (2-propyl-2-pentenoic, 2-propyl-3-penentioc, and 2-propyl-4-pentenoic acids) have been found in human plasma, each of them in concentrations >10% of the VPA concentration (1, 9).

The question of the clinical incidence of the observed difference between the two methods may be controversial. In our opinion, this difference is clinically acceptable in the sense that the VPA "therapeutic range" has not yet been sufficiently established and VPA concentrations are not closely related to its pharmacological effects (1).

We are grateful to Mrs. J. Genin-Ramakers for her skilled technical assistance.

References

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Table 1. VPA Found by EMIT and by GLC in Commercial Samples and in Two Pooled Human Drug-Free Sera Supplemented with VPA in Our Laboratory

<table>
<thead>
<tr>
<th></th>
<th>Expected</th>
<th>Found</th>
<th>VPA, mg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biotrol Therapeutique II 1a</td>
<td>50</td>
<td>50</td>
<td>49</td>
</tr>
<tr>
<td>Seronorm Pharmaca 402b</td>
<td>55</td>
<td>46</td>
<td>53</td>
</tr>
<tr>
<td>EMIT VPA Controlc</td>
<td>75</td>
<td>83</td>
<td>77</td>
</tr>
<tr>
<td>Seronorm Pharmaca 404b</td>
<td>130</td>
<td>117</td>
<td>128</td>
</tr>
<tr>
<td>A d</td>
<td>33</td>
<td>36</td>
<td>30</td>
</tr>
<tr>
<td>B d</td>
<td>88</td>
<td>86</td>
<td>88</td>
</tr>
</tbody>
</table>

Notes:
- a Batch 103, Laboratoires Biotrol, Paris, France.
- b Nyegaard & Co., Oslo, Norway.
- c Lot 6G 098-K01, Syva, Palo Alto, CA 94304.
- d Prepared in our laboratory.

These results are given in Table 1. No statistical difference between results by the two methods could be demonstrated. Nevertheless, the GLC values are in general closer to the expected values.

It appears that the EMIT calibrators (except for calibrator 150) contain something with a retention time in our GLC procedure that is very close to that of VPA. To completely resolve VPA and this substance, we assayed the EMIT calibrators on a SP 1000 phase with a temperature program (initial 180 °C; zero hold time; rate of rise 6 °C/min; final 220 °C). Under these conditions, the regression of our results on the values for supplemented samples has a slope of 1.04 (not significantly different from 1) and an intercept of −1.0 mg/L. When analyzed by our GLC method, the