background. Subsequently exposed to an ammonia atmosphere, the ochre spots turned blue, which rapidly faded back to a pale yellow in the absence of the ammonia. In our modification, in which NaOH was replaced by Na₂CO₃ thus permitting elimination of the subsequent exposure to ammonia, dark-blue color-stable spots on a pale-blue background were obtained. When protected from undue sunlight, the stained strips remained unchanged for as long as a year. This modified procedure also provides the same limit of detection (0.1-1.0 μg) of lipid as obtained in the original Jatzkewitz and Mehl method.

In our present chromatographic method, Eastman No. 6060 silica gel plates (20 x 20 cm) with fluorescent indicator were cut into 7.8 x 4.0 cm strips. The spotted test strips were first developed in hexane/ethanol/glacial acetic acid (80/20/1 by vol) for total lipid separation. A second development for phospholipid separation was with chloroform/methanol/distilled H₂O (65/25/4 by vol) (3). The strips were then removed from the micro cells, air dried, and placed into the BTB stain for about 1 min. After removal from the stain, the strips were dried with hot air.

Phospholipids were quantitated by elution with butylated hydroxytoluene in absolute methanol (50 mg/liter) and phosphorus determination content (4). Analytical recoveries averaged 84.3% (SEM ±2.73, n = 14).

References

Donald F. Yarmosh
Rose C. Fernandy

Miami Heart Institute
Research Division
4701 N. Meridian Ave.
Miami Beach, Fla. 33140

Evaluation of the Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT) Assays on the Technicon SMAC

To the Editor:

Results of performance studies on the SMAC are reported by Schwartz et al. (1) and Westgard et al. (2), but data on the assays of AST and ALT are given only by the latter with an old version of the computer software and before some important improvements in the methodology were introduced by the manufacturer. Before the introduction of these improvements, the results were not acceptable for routine analysis because of poor linearity and precision. We evaluated these assays on the SMAC after the introduction of an improved version of the computer software (version 7) and installation of several important improvements in methodology (new cartridge and new reagent composition). We used split samples to compare the SMAC and the Du Pont Automatic Clinical Analyzer (aca). The aca was calibrated on the values for patients’ samples that had been assayed by a manual method according to the Scandinavian recommendations (3).

Day-to-day variation for results with some different commercial lyophilized control sera on 40 consecutive working days, expressed in U/liter, was:

For AST, SMAC: mean = 32, SD = 3.8, CV = 11.9%; mean = 47, SD = 3.0, CV = 6.4%; mean = 83, SD = 2.3, CV = 2.8% for three sera.

aca: mean = 28, SD = 2.6, CV = 9.4%; mean = 108, SD = 3.2, CV = 2.9% for two other sera.

For ALT, SMAC: mean = 35, SD = 2.6, CV = 7.4%; mean = 113, SD = 4.1, CV = 3.6% for two sera.

aca: mean = 15, SD = 2.1, CV = 13.7%; mean = 137, SD = 3.1, CV = 2.3% for two other sera.

Westgard et al. (2) have recommended for both AST and ALT an allowable error (E₆) of 15% for an activity of 50 U/liter (E₆ = 7.5). The assays are acceptable if TE₆ < 7.5 and are not acceptable if TE₆ > 7.5. Our results are more or less in agreement with these criteria, especially if the systematic error is decreased by adjustment of the calibration.

We have used the assays for more than one year in clinical routine with satisfactory results.

References


G. J. H. Haan
F. R. Hindriks
A. Groen

University Hospital
Central Department for Clinical Chemistry
Groningen
The Netherlands

Determination of Carbamazepine in Serum

To the Editor:

Techniques for the estimation of carbamazepine were exhaustively discussed at a recent symposium on the reliable quantitation of antiepileptic drugs held in Kansas City, Mo. (November 29–December 1, 1976). Gas-liquid chromatography (GLC) remains the most popular technique for the estimation of these drugs, but estimation of carbamazepine by GLC does present problems. Underderivatized carbamazepine is thermally unstable and produces multiple peaks in different proportions during GLC separation (1, 2). To overcome this problem of thermal decomposition, several derivatization techniques have been proposed (1, 3, 4), but none has found wide acceptance. An alternative GLC procedure (5) does not seem to have attracted much attention in North America. In this procedure, the amide group of carbamazepine and of 10-methoxy-carbamazepine (used as internal standard) is converted to the cyanogroup. These products produce sharp peaks without any decomposition when analyzed by GLC.

Carbamazepine may also be estimated by thin-layer chromatography (TLC) (6, 7) or by high-performance liquid chromatography (8, 9). These techniques offer the advantage that derivatization is not required and thermal decomposition is not a problem. TLC has the advantage that many samples may be chromatographed simultaneously. For the last two years, we have been using the TLC procedure of Faber and Man In'T Weld (6), with minor modifications. We have found excellent correlation between results by this procedure and the GLC method of Gerardin et al. (5), using a nitrogen detector. The solvent system of this TLC procedure allows good separation of carbamazepine and its metabolites. These compounds become highly fluorescent after treatment with perchloric acid. Any of the metabolites, if required, may also be estimated on the same plate. Because both excitation and emission wavelengths of the fluorescent products are in the visible region, simple laboratory densitometers with glass optics may be used for measuring carbamazepine by this procedure.

We have participated in two international quality-control programs for the assay of antiepileptic drugs. Table 1 shows plasma carbamazepine values obtained by TLC compared with the target or mean values of these surveys for this drug. It appears that the TLC technique performs satisfactorily for the estimation of carbamazepine in plasma.

References


R. N. Gupta
Paul M. Keane

St. Joseph's Hospital
Hamilton, Ontario, Canada

Table 1. Quality Control Results for Carbamazepine

<table>
<thead>
<tr>
<th>Month (1976)</th>
<th>Found (TLC)</th>
<th>Target/mean value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sept.</td>
<td>8.0</td>
<td>8.0</td>
</tr>
<tr>
<td>Sept.</td>
<td>9.7</td>
<td>10.0</td>
</tr>
<tr>
<td>Sept.</td>
<td>6.4</td>
<td>6.0</td>
</tr>
<tr>
<td>Oct.</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Oct.</td>
<td>5.2</td>
<td>6.0</td>
</tr>
<tr>
<td>Oct.</td>
<td>2.3</td>
<td>3.0</td>
</tr>
<tr>
<td>Nov.</td>
<td>20.3*</td>
<td>21.8*</td>
</tr>
<tr>
<td>Nov.</td>
<td>11.4*</td>
<td>9.8*</td>
</tr>
</tbody>
</table>

* μmol/liter

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R. N. Gupta
Paul M. Keane
St. Joseph's Hospital
Hamilton, Ontario, Canada

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