Amino Acid Assay in “High-Performance” Analyzers

To the Editor:

Recent amino acid analyzers combine high-pressure technology and optimal elution conditions to speed the quantification of common amino acids in physiological fluids. However, operators must keep in mind that some infrequently observed amino acids may interfere with the accurate quantification of those that are clinically more relevant.

Although many interfering substances have been identified and their retention times characterized for systems using sodium citrate buffers (1), there is still very little information on retention times for the same substances chromatographed with the newer lithium-based systems (2).

We use a System 6300 High Performance Amino Acid Analyzer, with physiological buffers and elution parameters as supplied and recommended by the manufacturer (Beckman Instruments Inc., Palo Alto, CA). This system adequately separates the 39 amino acids present in the standard mixture supplied by the manufacturer.

Under these conditions, we have observed that glycine (R_{ty} = 0.088), the retention time relative to that of glycine, is insufficiently resolved to allow accurate estimation of urinary taurine peak areas (R_{ty} = 0.097). Similarly, we find that homocitrulline (R_{ty} = 1.453), excreted by infants on condensed milk feedings (8), co-elutes with methionine (R_{ty} = 1.448), making difficult the detection of clinical hypermethtioninuria.

When authentic penicillamine is run, the sulphydryl form elutes at R_{ty} = 0.911 and obscures the proline peak (R_{ty} = 0.942). Penicillamine disulfide elutes at R_{ty} = 1.495; the peak overlaps methionine (R_{ty} = 1.481) and cystathionine (R_{ty} = 1.509) standards and follows closely on the cystine peak (R_{ty} = 1.464). In cystinuric patients who are receiving therapy with penicillamine, the cysteine—penicillamine mixed disulfide and penicillamine disulfide are not resolved from cystine, thus making it impossible to determine the effectiveness of treatment.

Finally, in two phenylketonuric sera, we have observed a spurious peak (R_{ty} = 1.698), which may be cysteinylglycine (4). It causes shouldering of the phenylalanine peak (R_{ty} = 1.741) and makes quantification of serum phenylalanine less accurate.

We would like to encourage the reporting of similar observations. We would also welcome the publication of programs that obviate these problems or any others encountered by users of this system.

References

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On the Stability of EMIT Assay Curves: Contradictory Observations

To the Editor:

Bach and Larsen (Clin Chem 26: 652–654, 1980) previously made the following observations regarding the stability of standard curves prepared for EMIT Homogeneous Immunoassay Kita (Syva Co., Palo Alto, CA) stored at room temperature:

- Absorbance changes for all calibrators, including the zero calibrator, increased gradually with time.
- Increases of non-zero calibrators were largely affected by a parallel increase in the zero calibrator, such that the quantity \( \Delta A - \Delta A_0 \) was very nearly constant with time.

Use of standard curves based on the quantity \( \Delta A - \Delta A_0 \) can be extended from 24 h to 18 days (for procainamide) by correcting all \( \Delta A \) values with a new \( \Delta A_0 \).

In the September 1983 issue of Clinical Chemistry (29: 1690, 1983), Gorsky, from the same laboratory, reports in a letter that the EMIT assay curves are stable for at least a week. He attributes his findings to automation of sample- and reagent-handling steps and the use of the CP-6000 and two-point calibration in place of the CP-5000 and six-point calibration. Gorsky claims that results produced for seven to eight days from a single assay calibration curve are satisfactory with no calibration adjustment or recalibration.

Automation of sample- and reagent-handling steps can only improve precision; it cannot correct for drift that is reagent-related. Regardless of whether the calibration curve is produced by the CP-6000 or the CP-5000, changes in the \( \Delta A \) of reactions will be reflected in the results, so that an increase in the \( \Delta A \) of a test reaction will give a higher result. The data presented by Gorsky support his claim but contradict those of Bach and Larsen, and vice versa. These two observations from the same laboratory cannot be both correct. Either one is or is not a gradual increase in the \( \Delta A \) of calibrators with time.

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Authors of the above-cited communications respond:

To the Editor:

Unfortunately, Repique and Farber overlooked some critical aspects of the two papers they cite. Bach and Larsen noted that data used in preparing the standard curve changed most rapidly during the first 48 h after reagent reconstitution. After that time, there was a plateau period of about a week (see Table 1 of their paper). By allowing the reconstituted reagents to stand for 72 h before use, Gorsky avoided the initial rapid period of change in order to work within the plateau phase.

If one is familiar with the manual steps involved in the procedure involv-