Immediately after removal from the oven, the thin-layer chromatographic plate is immersed in dodecane (Aldrich no. D 22, 110-4; n\textsuperscript{D} = 1.4212, approximately that of silica) in a small vacuum desiccator. By reducing the pressure, the air trapped in the plate foams out and on return to ambient pressure, the plate becomes almost transparent.

The plate is scanned at 470 nm, beam mask 5 x 2 mm, in a scanning-integrating densitometer (Transidyne Model no. 9280). Typical data are given in the following tabulation:

<table>
<thead>
<tr>
<th>Standard ratios</th>
<th>Gravimetric</th>
<th>Densitometric</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/1</td>
<td>2/1</td>
<td>3/1</td>
</tr>
<tr>
<td>4/1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a Pooled ranges from 10 runs. Within-run ratios show consistent differences.

The observed L/S ratios for standards generally are less than the theoretical values, but show a regular and reproducible progression that permits interpolation of the values of the unknowns. If present, phosphatidyl inositol lies between lecithin and sphingomyelin; phosphatidyl serine and phosphatidyl ethanolamine migrate beyond the lecithin band and separate distinctly from it. Lysolecithin barely migrates away from the origin. Interestingly, the copper sulfate/phosphoric acid reagent is not darkened by synthetic lecithin (palmitoyl or stearoyl), suggesting that the reaction is a reduction of copper by unsaturated fatty acids in natural lecithin and sphingomyelin, which must be used as standards. About 2.5 h is required to perform this procedure from receipt of specimen to evaluation. A copy of the test protocol is available on request. The plates are evaluated as follows: L/S ratio <2/1 "suggests fetal immaturity"; >2/1 but <3/1 "suggests borderline zone"; and >3/1 "suggests fetal maturity." These ratios were set on the basis of our clinical experience.

References

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**Enzyme Immunoassay, with Use of a Centrifugal Analyzer, of Phenytoin, Phenobarbital, and Theophylline**

To the Editor:

Recently, Haven (1) and London et al. (2) described adaptation of the "Enzyme Multiplied Immunoassay Technique" (EMIT; Syva, Palo Alto, Calif. 94304) for phenytoin and phenobarbital to the Centrifichem (Union Carbide, Rye, N. Y. 10580). We describe here a modified method for the assay of phenytoin and phenobarbital, which also is applicable for theophylline determinations.

This modification deletes the manual pipetting of Reagent A and avoids the addition of water during the sample flush. The Sample Diluent Pump on the Centrifichem is emptied, dried, and then filled with diluted Reagent A from the Diluent Reservoir. The remaining Reagent A in the reservoir is then returned to the reagent bottle. In this manner Reagent A is added automatically as the diluent flush following the sample.

During pipetting the Diluent Reservoir filled with distilled water acts to rinse the pipette tip between sample aspirations. Upon completion of pipetting, residual Reagent A is aspirated from the pump and returned to the reagent bottle.

These modifications eliminate the need for prior dilution of standards or samples as previously reported by London et al. (2). The sample size meets instrument specifications and avoids sample volumes of less than 5 µl as suggested in a previous method (1).

All reagents, samples, and buffers are in the same proportion in this method as in the recommended EMIT procedure. Reagents are prepared as per kit instructions except as follows:

- **Reagent A**—reconstitute with 6.0 ml of distilled water. **Working Reagent A**—to one volume of stock Reagent A add 1.3 volumes of EMIT Buffer Solution. This reagent is used to fill the Sample-Diluent Pump.
- **Reagent B**—reconstitute with 6.0 ml of distilled water. **Working Reagent B**—to one volume of Reagent B add 14.9 volumes of EMIT Buffer Solution. (This final volume is sufficient for full rotor but may be decreased proportionately for fewer samples.)

**Pipettor setting**: reagent volume, 350 µl; sample volume, 5 µl; total volume, 55.0 µl.

**Analyzer test parameters**:

- \( T_0 = 10 \text{ s} \)
- \( \Delta T = 15 \text{ s} \)
- Abnormal absorbance = 2.0
- Blank = Auto

Test mode = Terminal
Printout = Absorbance
Number of prints = 9

Results are calculated by plotting the \( \Delta A \) from prints one and six. Lot-specific graph paper provided with each kit is used. The within-run CV averages 2.5% or less. The inter-run CV ranges from 4.0 to 5.0% within the therapeutic range.

References

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**More on Use of Hair in Trace-Metal Analysis**

To the Editor:

I wish to comment upon the interesting and informative paper of Assarian and Oberleas (1).

Obviously it is impossible, from analysis of untreated hair, to state categorically which proportion is "true" metal content and which is present as a contaminant. However, it is usually possible to make an intelligent assessment of the probability of contamination.

In these authors' paper, the marked loss of magnesium can be simply explained. Because this element is present in water and readily forms insoluble soaps, it is very probable that magnesium was lost simply by removal of the magnesium soaps on the hair during the washing procedure. Furthermore, this particular example is not a practical problem, because it seems unlikely that hair analysis would be used in the case of this element.