percentage of the lipase activity, are apparently attributable to the pseudolipase removal from sera which contain both pancreatitis lipase and pseudolipase. An example of this is included in Table 1.

On the other hand, the cause for the occasional slight increases in the assay value due to MCE treatment is unclear. Random errors were eliminated as a possible cause by the experimental design. Another possible cause is reactivation of reversibly inactivated lipase. A third possibility is modification of lipoproteins by MCE so that clearing of the substrate is facilitated during the reaction. These increases are slight and do not appear to interfere with the diagnostic usefulness of the procedure.

The odor of MCE is one of its disadvantages. Consequently, we tried three other sulfhydryl compounds under similar conditions: preincubations for 6 min at 25 °C at an inhibitor concentration of 0.1 mol/liter. Reduced glutathione partly inhibited (about 50–70% inhibition) both pancreatitis lipase and pseudo-lipase activities. Dithiothreitol caused essentially complete inhibition of pseudolipase activity, but also significantly inhibited pancreatitis lipase. Cysteine caused complete inhibition of both pancreatitis lipase and pseudolipase activity in all specimens tested. Hence, of those tried, MCE was the best reagent for differentiating pancreatitis lipase and pseudo-lipase.

Table 1 gives representative data illustrating the effectiveness of this MCE preincubation in removing pseudolipase activity. The MCE treatment indicated above was followed: A 0.2 mol/liter MCE solution was prepared just before use by dissolving 70 μl of MCE in 5 ml of 8.5 g/liter NaCl. Equal volumes of serum and 0.2 mol/liter MCE were mixed in a small tube and allowed to stand at room temperature for 6 min. Lipase was then measured in 0.05 ml portions of this two-hour dialysis solution according to the directions given by the Perkin-Elmer Corp. for serum analysis with the Amylase–Lipase Analyzer. If necessary, as with high pancreatitis lipase activity, we further diluted the serum in 0.2 mol/liter MCE in order to obtain meter readings between 1 and 3 kU/liter on the instrument. Amylase was measured with the Amylase–Lipase Analyzer according to the directions given by the Perkin-Elmer Corp. Amylase and the Tietz–Fiereck (1) lipase assay values are included as evidence for or against pancreatic involvement. The normal ranges applying here are: nephelemetric lipase, 0–1.6 kU/liter; Tietz lipase, 0–1.0 kU/liter; and nephelometric amylase, 150–2000 U/liter.

References

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Ethanol As a Mobile-Phase Solvent in High-Performance Liquid-Chromatographic Assays

To the Editor:

To date, acetonitrile and methanol are probably the most popular water-miscible organic solvents used in mobile phases of high-performance liquid chromatography, special grades of them available from various manufacturers being ordinarily used. They are usually glass-distilled and of very high purity, and so they are expensive. In our laboratory the much less expensive alcohol USP (96% ethanol) has been extensively used in the last year in lieu of acetonitrile or methanol for assays of many drugs in plasma, such as theophylline, chloramphenicol, salicylates, acetaminophen, ampicillin, and sulfisoxazole. The results have been very satisfactory.

In view of its economy and apparent unpopularity among other workers it seems worthwhile to report here our experiences.

We evaluated the feasibility of using alcohol USP in this technique by comparing its ultraviolet absorption profile with that of acetonitrile and methanol, both of high-performance liquid-chromatographic grade. Their ultraviolet absorption (vs. distilled water) was studied in a spectrophotometer with a recorder and digital readout meter (UV-Vis Spectrophotometer, Model 200; Perkin-Elmer Corp., Norwalk, Conn.). Their absorbances at six selected wavelengths are summarized in Table 1. The acetonitrile showed the best ultraviolet light transparency, but the alcohol USP appears to be equally as good as methanol from one source and to excel from the other. The greater absorption of ethanol as compared to acetonitrile did not appear to affect the performance of our system. For example, the baseline from a mobile phase of alcohol USP/acidified water (pH = 2.5, 20/80 by vol, and reversed-phase column (μ-Bondapak C; Waters Associates, Inc., Milford, Mass.) is stable, with little noise. We have used this system successfully to assay salicylates, theophylline, and ampicillin in plasma by monitoring their ultraviolet absorption at 237, 275, and 212 nm, respectively. For the mobile phase containing water, there is no need to use absolute ethanol (reagent or USP grade) even though it is also reasonably ultraviolet transparent.

In view of the purity and suitability of ethanol to be used as a water-miscible component in mobile phases and its relative cheapness, we recommend that 95% or absolute ethanol should be first tried as a possible solvent in developing high-performance liquid-chromatographic assays.

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Table 1. Absorbances (A) of Acetonitrile, Methanol, and Ethanol Compared

<table>
<thead>
<tr>
<th>Component</th>
<th>A at 350 nm</th>
<th>325 nm</th>
<th>300 nm</th>
<th>254 nm</th>
<th>225 nm</th>
<th>210 nm</th>
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</thead>
<tbody>
<tr>
<td>Acetonitrile</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Methanol</td>
<td>0.002</td>
<td>0.002</td>
<td>0.001</td>
<td>0.006</td>
<td>0.039</td>
<td></td>
</tr>
<tr>
<td>Ethanol</td>
<td>0.005</td>
<td>0.020</td>
<td>0.190</td>
<td>0.550</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ethanol, 95%</td>
<td>0.005</td>
<td>0.020</td>
<td>0.190</td>
<td>0.554</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ethanol, absolute</td>
<td>0.011</td>
<td>0.035</td>
<td>0.320</td>
<td>0.636</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- Glass-distilled; Burdick and Jackson Laboratory, Muskegon, Mich.
- HPLC-grade; Fisher Scientific Co., Fair Lawn, N. J.
- Alcohol, USP; Medical Center General Stores, University of Illinois at the Medical Center, Chicago, Ill.
- Alcohol, dehydrated; USP, Medical Center General Stores, University of Illinois at the Medical Center, Chicago, Ill.