Table 2. Retention Times for Some Psychiatric Drugs Relative to Haloperidol

<table>
<thead>
<tr>
<th>Drug</th>
<th>Retention time Relative to haloperidol</th>
<th>Min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haloperidol</td>
<td>1</td>
<td>2.9</td>
</tr>
<tr>
<td>Perphenazine</td>
<td>2.72</td>
<td>7.9</td>
</tr>
<tr>
<td>CI-Haloperidol</td>
<td>1.72</td>
<td>5.0</td>
</tr>
<tr>
<td>Thioridazine</td>
<td>1.51</td>
<td>4.4</td>
</tr>
<tr>
<td>Fluphenazine</td>
<td>1.1</td>
<td>3.2</td>
</tr>
<tr>
<td>Flupenthixol</td>
<td>1.1</td>
<td>3.2</td>
</tr>
<tr>
<td>Chloridiazepoxide</td>
<td>1.1</td>
<td>3.2</td>
</tr>
<tr>
<td>Chlorpromazine</td>
<td>0.89</td>
<td>2.6</td>
</tr>
<tr>
<td>Trihexyphenidyl</td>
<td>0.37</td>
<td>1.1</td>
</tr>
<tr>
<td>Trifluoperazine</td>
<td>0.31</td>
<td>0.9</td>
</tr>
<tr>
<td>Diazepam</td>
<td>0.31</td>
<td>0.9</td>
</tr>
<tr>
<td>Desimipramine</td>
<td>0.18</td>
<td>0.55</td>
</tr>
<tr>
<td>Nortryptiline</td>
<td>0.17</td>
<td>0.5</td>
</tr>
<tr>
<td>Imipramine</td>
<td>0.13</td>
<td>0.4</td>
</tr>
<tr>
<td>Amitryptiline</td>
<td>0.13</td>
<td>0.4</td>
</tr>
<tr>
<td>Trihexyphenidyl</td>
<td>0.13</td>
<td>0.4</td>
</tr>
<tr>
<td>HCl Pimozide</td>
<td>not eluted</td>
<td></td>
</tr>
</tbody>
</table>

was 1 ng. The actual sensitivity of the method when 5 mL of plasma was extracted was 0.5–1 μg/L.

The specificity of the method is indicated by the characteristic gas-chromatographic retention times of the reference compounds and the lack of interfering peaks in plasma controls not treated with haloperidol. Table 2 lists these values for haloperidol and the internal standard, and for some drugs that might interfere in the assay.

This method is straightforward—no derivatization of haloperidol being necessary—and is reproducible, as shown by the lower interassay CV, two assets that make it very suitable for routine clinical laboratory use. Drugs that might be given to patients in association with haloperidol such as tricyclic antidepressants, benzodiazepines, and phenothiazines did not interfere with the analysis.

Much of the reproducibility of this assay must be attributed to the use of the NPD. An ECD contaminated by impurities in an injection may take a long time to recover its previous baseline and subsequent sensitivity; with the NPD, however, even fairly large injection volumes may be put through without disrupting its performance.

The method I describe offers the following advantages over other published gas-chromatographic and radioimmunoassay methods: (a) an extra evaporation step required in the method described by Bianchetti and Morselli (2) is more time consuming, and may lead to loss of drug because of adsorption and may concentrate solvent impurities; (b) the use of n-heptane and n-hexane for the extraction from plasma lowers the signal-to-noise ratio on the chromatogram, because fewer and less unwanted impurities are extracted than when diethyl ether is used for extraction; (c) an NPD is used for detection, as discussed above; (d) because of the sensitivity of the NPD, relatively small volumes of plasma are required; (e) the method is sufficiently sensitive for pharmacokinetic studies in subjects receiving standard clinical doses in which specificity is an advantage; and (f) the reagents are more easily acquired than those for radioimmunoassay methods. The advantage that radioimmunoassay techniques have over other existing methods is that they require less plasma and, in general, no prior extraction of the drug from the plasma before assay is required.

The method described here requires plasma sample volumes of 0.5–5 mL, depending on the patient’s dosage. It is sensitive, specific, reproducible, and procedurally suitable for routine clinical use.

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References

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Indoxyl Sulfate Interferes with Dip-and-Read Urinary Bilirubin Estimate

To the Editor:

Interferences in the diazo determination for urinary bilirubin are widely recognized. In an effort more precisely to characterize the cause of these interferences, we examined 370 bilirubin-negative urines obtained from hospitalized patients for appearance of interfering color in the bilirubin test with Ames N-Multixat®. We found that 52 of these urines (14%) produced an atypical visual response (an orange to red-orange color not appearing on the color chart). Only three of 79 urines from non-hospitalized individuals (4%) produced this response.

Several of the atypical urines were pooled and analyzed by thin-layer silica gel chromatography in both acid (n-butanol/acetone/acetic acid/water, 7/7/2/4 by vol) and basic (isopropanol/ammonium hydroxide/water, 40/5/5 by vol) solvent systems. After development, chromatograms were sprayed with modified Ehrlich reagent (1) or diazotized 2,4-dichloroaniline. Colored spots were seen that migrated to about the same relative locations as indoxyl sulfate. Rf values were .56 in the acidic solvent, .75 in the basic solvent.

We confirmed the identity of indoxyl sulfate as the component responsible for the atypical color by the Fluorinal Reaction test (1) and by acid oxidation (of indoxyl sulfate to indigo) with use of potassium persulfate in concentrated HCI (1). This finding has been alluded to in a previous note (2).

We subsequently analyzed 24 individual atypical color-producing urines by thin layer chromatography and found that in 21 of these urines, indoxyl sulfates were the cause of atypical color. Thus, for more than 85% of the atypical urines studied the interference observed apparently was ascribable to this compound.

Jirsa and Jirsová (3) reported indoxyl sulfate as a color-distorting compound in urines found by diazo assay to be positive for bilirubin. Our results extend this finding with the observation that indoxyl sulfate produces color of its own in urines that are negative for bilirubin, and indeed was found to be the causative agent in the vast majority of atypically colored urines tested in our study. We also confirm Jirsa and Jirsová’s finding for bilirubin-positive urines: the presence of indoxyl sulfate in bilirubin-positive urines can alter the dip-and-strip color sufficiently to mask a weak bilirubin reaction. Further, analysis of some bilirubin-positive urines in our laboratory revealed substantial indoxyl sulfate concentrations in as many as half of them. Therefore, we emphasize the importance of using a more nearly specific confirmatory test (such as Ictotest®) whenever dip-and-read tests for bilirubin are inconclusive.

References
3. Jirsa, M., and Jirsová, V., Močový indikán
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Determination of High-Density Lipoprotein Cholesterol by Heparin–MnCl₂ Precipitation

To the Editor:

Precipitation with heparin–MnCl₂ has been used for 20 years to remove apolipoprotein-B-associated lipoproteins (low- and very-low-density lipoproteins) from plasma (1). Since 1974 (2), the technique has been accepted as a standard investigative tool. In the conventional method, low- and very-low-density lipoproteins (LDL and VLDL) are precipitated at a final concentration of 46 mmol/L of MnCl₂ · 2 H₂O. The recent papers by Warnick and Albers and their co-workers (3, 4) recommend that the final concentration of MnCl₂ be increased to 92 mmol/L, because this results in values that agree closely with measurements of high-density lipoprotein (HDL) cholesterol done on HDL isolated from plasma by ultracentrifugation. In addition, their previous experience with this modification demonstrated that it decreases the frequency of turbid supernates and there is less apolipoprotein-B-associated cholesterol in the clear supernates.

As shown in Figure 1, we have compared the conventional and recommended methods at a final MnCl₂ concentration of 46 mmol/L and 92 mmol/L separately. At a final concentration of 46 mmol/L of MnCl₂, it is obvious that VLDL- and LDL-cholesterol precipitations are incomplete. Previous evidence (3, 4) that HDL-associated cholesterol is not precipitated by this modification of the MnCl₂ concentration has been limited to a failure to find an increased amount of apolipoprotein A-I, a major apoprotein which constitutes 60% by weight of HDL protein (5), in the washed precipitates as measured by radial-immunodiffusion assay (3, 4).

To support further the utility of using this recommended concentration of MnCl₂ for determination of HDL-cholesterol, we measured apolipoprotein A-I in the supernates of samples in which LDL and VLDL were precipitated with heparin and various concentrations of MnCl₂ (0–160 mmol/L), using a recently described sensitive radioimmunoassay (5). After centrifugation, the supernates were delipitated with ether/ethanol and diluted to 20 000 fold before subjecting them to the double-antibody radioimmunoassay procedure. As demonstrated in Figure 1, increasing the concentration of MnCl₂ had no effect on the concentration of apolipoprotein A-I in the supernates, but cholesterol in LDL and VLDL was precipitated, suggesting that HDL is not co-precipitated by this technique.

Thus, our studies support the procedure recommended by Warnick and co-workers and we would recommend its use as a standard method for measuring HDL-cholesterol in plasma.

References


Multiple Venous Samples: A Cautionary Tale

To the Editor:

Most of our extensive knowledge of human blood chemistry is based on analysis of samples collected from the antecubital veins; there is little information about the chemical composition of venous blood taken from other sites. Recently, a lady attending this hospital for investigation of progressive hypertension was subjected to a venogram, and the laboratory received six venous samples taken from different sites in the course of that procedure.

The blood was collected in heparinized tubes containing plastic beads, which have a relative density intermediate between that of normal plasma and erythrocytes. After centrifugation of the samples, we noted that the beads were floating on the plasma in the sample taken from the right renal vein. Preliminary investigations and enquiries indicated that this sample was contaminated with contrast medium. For the venogram, sodium iohalamate (700 g/L solution) and meglumine iohalamate (600 g/L) had been used in various amounts. There was no assay method for these substances available, so a crude estimate was attempted by analyzing each sample for its major constituents, and summing the total volumes.

Plasma water was measured by titration with Karl Fischer reagent (1) and total lipid was measured by the phospholsofuoranilic method; the other analyses were by standard procedures. The various methods were checked for interference by the contrast medium. Sodium iohalamate increased the sodium reading, and at 20 mL/L it increased the flame-photometric potassium reading by 10%. Both media decreased the color yield in the lipid assay. The degree of inhibition was proportional to the amount of medium present, the relation being expressed by the following equations: log concn (mL medium per 100 mL) = 0.0244 % inhibition – 0.9402 ± 0.0168 and % inhibition = 40.82 log concn (mL medium per 100 mL) + 38.45 ± 0.688, with r² equal to 0.9984.

The analyses for aldosterone and renin were performed by a reference