that specified before (1), reflecting the lower purity of the commercial preparation.

For calculation of the P-type isoamylase activity, we selected the linear portion of the reaction-rate curve as determined from a strip-chart recorder. The curve was most often linear between 7 and 10 min after the reaction was begun, at which time deviations from linearity are minor but increase with time. Such deviations from linearity may be due to a dissociation of the enzyme–inhibitor complex, resulting in a gradual increase in enzyme activity (1).

Results

We determined optimal inhibitor concentration by adding various concentrations of S-type amylase inhibitor to appropriate dilutions of pancreatic extract and saliva and assaying. Optimal concentrations of the S-type inhibitor as prepared by Sigma and the inhibitor as prepared by O’Donnell et al. inhibited the S-type amylase fraction by 86 to 88%, but the P-type fraction by only 16 to 20%. That is to say, the remaining P-type amylase activity on using either of the two inhibitors was 80 to 84% of the original, while only 12 to 14% of the S-type amylase activity was retained.

This experiment suggests that the two inhibitors of S-type amylase are comparable. To examine this further, we did experiments on specimens from patients with hyperamylasemia resulting from various clinical conditions, predominately pancreatitis. Table 1 shows that results obtained with the two inhibitors were virtually identical (P-type activity, \( r = 0.9999 \); S-type activity, \( r = 0.9998 \)).

References

1. Huang WH, Tietz NW. Determinations of amylase isoenzymes


Improved Liquid-Chromatographic Determination of Haloperidol in Plasma

Amyla K. Dhar and Henn Kutt

This method for determination of haloperidol in plasma is based on "high-performance" isocratic liquid chromatography with the use of a C8 bonded reversed-phase column at room temperature. Haloperidol and the internal standard (chloro-substituted analog) are extracted from alkalized plasma into isoamyl alcohol/heptane (1.5/98.5 by vol) and back-extracted into dilute H2SO4. The aqueous phase is directly injected onto the column. The mobile phase is a 30/45/25 (by vol) mixture of phosphate buffer (16.5 mmol/L, pH 7.0), acetonitrile, and methanol. Unlike other liquid-chromatographic procedures for haloperidol, commonly used psychotropic drugs do not interfere. Analysis can be completed within an hour. The procedure is extremely sensitive (1.0 \( \mu \)g/L) and is well reproducible (CV 5.6% for a 2.5 \( \mu \)g/L concentration in plasma).

Additional Keyphrases: chromatography, reversed phase, drug assay, psychotropic drugs, urine

Haloperidol, 4-(4-(4-chlorophenyl)-4-hydroxy-1-piperidinyl)-1-(4-fluorophenyl)-1-butane, is widely used in the treatment of acute and chronic psychiatric and neurological disorders. The pharmacokinetics, including the metabolism of haloperidol and its distribution in tissue, have been extensively studied (1, 2). Studies on the therapeutic as well as the toxic effects of haloperidol reveal a positive correlation between the concentration of haloperidol in plasma and its clinical effects (3–5). Thus children with Guilles de la Tourette syndrome improved when their haloperidol concentration in plasma was 1 to 4 \( \mu \)g/L, while in psychiatric syndromes concentrations of 6 to 12 \( \mu \)g/L were needed. Side effects commonly start to appear when concentrations approach 10 \( \mu \)g/L.

Unfortunately, no simple procedure is available for determining haloperidol in plasma in this range of concentrations. Several methods reported, including gas-chromatography with nitrogen–phosphorus or electron-capture detector (6–8) and radioimmunoassay (9, 10), are relatively time consuming and impractical for use in routine monitoring of the drug.

Recently, several liquid-chromatographic procedures (11–14) have been described for determining haloperidol in plasma. In that of Jatlow et al. (11), the internal standard is desipramine, the most widely prescribed antidepressant drug. Other commonly prescribed tricyclic antidepressants,
e.g., imipramine, amitriptyline, nortriptyline, and doxepin, would be expected to interfere in the assay. Benzodiazepines and phenothiazines reportedly also interfere in the assay, as well as a commonly prescribed cold medication, diphenhydramine. Those authors tried three different columns (C18, C8, and C6) and recommended that one or the other be used depending on what interfering drugs were expected (from the patient's drug history) to be present in the sample. These factors limit the usefulness of that procedure for clinical and research laboratories.

Another report (12) describes reversed-phase chromatography with electrochemical detection of haloperidol and its reduced metabolite in plasma. The reduced metabolite of haloperidol is reportedly (12) 5 to 25% as potent as haloperidol but is not detected in liquid chromatography with detection by ultraviolet absorption (11, 13, 14). Ultraviolet absorbance at 254 nm of the reduced haloperidol is diminished totally, owing to the loss of carbonyl chromophoric group. The reduced metabolite (an alcohol) is also more polar than the parent drug (a keto alcohol) and has a lower retardation factor (K') (12). A recent report (10) suggests that a high ratio of reduced haloperidol to haloperidol may correlate with poor clinical response, a possibility that needs further investigation.

In another reported procedure (13) no internal standard was used, and a heated column was required. The most recently reported procedure (14) also lacks simplicity and is not very sensitive, only half of the final extract being injected into the chromatograph. This procedure also is likely to be susceptible to interferences from all commonly used anti-depressant and psychotropic drugs, because the elution time for haloperidol and the internal standard is very short when the heated column is used.

We describe here a rapid, simple procedure based on liquid/liquid extraction of the drug and the internal standard, with subsequent isocratic reversed-phase liquid chromatography with ultraviolet detection at 254 nm, at ambient temperature. The procedure is highly sensitive, and requires only commonly used isocratic liquid-chromatographic apparatus and a sensitive fixed-wavelength ultraviolet detector.

Materials and Methods

Materials. Haloperidol (Haldol) and its chloro-substituted analog were a gift from McNeil Laboratories, Fort Washington, PA 19034. We used this analog as the internal standard in procedure. All reagents and water were "HPLC" or reagent grade.

A Beckman Ultrasphere (Beckman Instruments, Inc., Berkeley, CA 94710) C8 (5-µm) 25 cm × 4.6 mm (i.d.) reversed-phase column was used for the analyses. We used a Beckman Model 331 pump equipped with an Altex injection valve with a 500-µL loop and attached to a Model 773 Spectro-monitor ultraviolet detector (Kratos Analytical Instruments, Ramsey, NJ 07446). The sensitivity setting for the Kratos detector was 0.005 A full scale at 254 nm. Alternatively, we used an HP 5880 (Hewlett-Packard, Paramus, NJ 07652) liquid chromatograph equipped with a fixed-wavelength (254 nm) detector and a HP 3390 integrator recorder.

The mobile phase was prepared by mixing 300 mL of Na2HPO4 (16.5 mmol/L, pH 7.0) with 450 mL of CH3CN and 250 mL of methanol and filtering through 0.45-µm pore-size HA-filters (Millipore, Bedford, MA 01730).

Preparation of plasma-based standards and calibrators. Haloperidol (10 mg) and the internal standard (10 mg) each were dissolved in 100 mL of methanol (stock). When stored at 4 °C, these stock solutions were stable for at least six months. To prepare haloperidol standards in plasma, we diluted the stock solution with drug-free plasma to yield concentrations of 2.5, 5.0, 10, 25, and 50 µg/L. A 25 µg/L plasma standard was always used as control. The standards and control-plasma samples were stable for at least six months when stored frozen at −20 °C. The working solution of internal standard was 50 µg/L in water.

Extraction procedure. To 0.5 to 1.0 mL of plasma sample or standard placed in a 15-mL screw-capped tube, add 0.5 mL of the internal standard solution (50 µg/L) and 0.5 mL of 1 mol/L NaOH; vortex-mix. Add 5 mL of a solution of 15 mL of isooamyl alcohol per liter of heptane, shake each tube for 5 min, and centrifuge. Transfer the solvent layer to a screw-capped tube containing 0.6 mL of 5 mmol/L H2SO4, shake for 5 min, and centrifuge for 7 min at high speed. Carefully aspirate the solvent layer and inject 0.4–0.5 mL of the aqueous layer onto the column.

Calibration. Prepare a standard curve by plotting the ratios of the peak heights of haloperidol and the internal standard against the concentration of haloperidol; calculate the concentration of haloperidol from the inverse of the slope of this curve. Alternatively, we read the concentration directly from the integrator printout.

Results and Discussion

Figure 1 shows typical chromatograms of drug-free plasma, a drug standard in plasma, and plasma and urine from a patient receiving haloperidol. The peaks of interest are

Fig. 1. Typical chromatograms of (1) haloperidol, and (2) chloro-haloperidol
(a) drug-free plasma extract; (b) plasma containing haloperidol, 50 µg/L, and internal standard, 25 ng; (c) plasma of patient receiving haloperidol, extracted after internal standard was added; and (d) urine sample from the same patient, with added internal standard, extracted and analyzed as in c
symmetrical and are eluted in less than 10 min. We have noted no interference from other commonly used psychoactive drugs, such as phenothiazines and their metabolites. Antidepressants, such as amitriptyline, imipramine, doxepin, trimipramine, trazodone, and maprotiline (and their metabolites, including their hydroxylated metabolites), although extracted by this procedure, are eluted within the first 3 min. Some phenothiazines, such as fluphenazine and chlorpromazine, elute later (12 min). Amoxapine, a recently introduced antipsychotic drug, also has a longer retention time (12 min). Alkalization of the plasma before extraction apparently prevents co-extraction of acidic drugs.

The calibration curve is linear over the range of drug concentrations used (2.5 to 50 μg/L, n = 8) and passes through the origin, with a correlation coefficient of 0.9996.

Analytical recoveries of added haloperidol from plasma by this extraction procedure (mean ± SD, n = 8) were 89 ± 2% and 88 ± 3% at concentrations of 10 and 25 μg/L, respectively.

Reproducibility of extraction of the drug from plasma was within acceptable limits (Table 1). The limit of detection is 1 μg/L. Routine determinations by the present procedure of haloperidol in plasma from a large number of patients receiving different dosages of the drug and various comediations varied from <1.0 to 50 μg/L.

The present procedure for the determination of haloperidol is more sensitive and less cumbersome than most previously described methods. The procedure is practical for measuring haloperidol concentrations in plasma from patients who are receiving chronic dosages of haloperidol with or without additional commonly prescribed psychoactive drugs.

Liquid-chromatographic procedures have distinct advantages over other methods described previously (6–10). Gas-chromatographic procedures for haloperidol assay are encumbered by the drug's high temperature of vaporization. The procedure described here is easily set up in any laboratory equipped with liquid chromatographs having a sensitive fixed- or variable-wavelength detector. Unlike other liquid-chromatographic procedures published previously (11–14), this procedure is carried out at ambient temperature. The sensitivity of the procedure makes it especially suitable for analysis of small volumes of plasma, such as from pediatric patients. Finally, the method can potentially be used to determine other butyrophenone drugs.

References

Table 1. Precision and Accuracy of Determination of Haloperidol in Plasma

<table>
<thead>
<tr>
<th>Haloperidol, μg/L</th>
<th>Expected (mean ± SD, n = 8)</th>
<th>Measured CV, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.5</td>
<td>2.48 ± 0.14</td>
<td>5.6</td>
</tr>
<tr>
<td>5.0</td>
<td>5.16 ± 0.44</td>
<td>8.5</td>
</tr>
<tr>
<td>10.0</td>
<td>10.13 ± 0.47</td>
<td>4.5</td>
</tr>
<tr>
<td>25.0</td>
<td>25.20 ± 1.09</td>
<td>4.3</td>
</tr>
<tr>
<td>50.0</td>
<td>50.20 ± 1.91</td>
<td>3.8</td>
</tr>
</tbody>
</table>