Determination of Promethazine in Serum by Liquid Chromatography

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We describe a procedure for the liquid-chromatographic determination of promethazine in low-nanogram concentrations in serum. Trifluromazine is used as the internal standard. The method is based on a single extraction of promethazine from serum with hexane and subsequent derivatization with trichloroethyl chloroformate. Analytical recovery of promethazine is about 90%. The lower limit of detection is 1 µg/L when a 2.0-mL aliquot of serum is assayed. Our data illustrate the practicability of the method for bioavailability studies after oral or rectal administration of promethazine hydrochloride.

Additional Keyphrases: determination of phenothiazine compounds • drug assay • monitoring therapy • pharmacokinetics

Promethazine, a phenothiazine drug, is widely used as an antihistamine and a mild sedative. Little is known about its disposition in man (1). Very few methods have been reported for quantitative determination of the drug in biological specimens. The instability of promethazine in aqueous and organic solvents (2) and the low concentrations in serum associated with its therapeutic use in humans principally account for the difficulty in developing methods for its determination in serum. Gas-chromatographic methods with flame-ionization detection are applicable only to toxic concentrations of the drug in serum (3–5). A substantial increase in sensitivity is achieved by gas-chromatographic methods in which nitrogen-specific detectors are used (6–8). Such methods can be used to determine high therapeutic concentrations of promethazine in blood and urine (7), but they can detect no less than 5 to 10 µg/L, even under ideal analytical conditions (8). For bioavailability studies and the therapeutic monitoring of promethazine concentrations, it is mandatory that the method be specific and be considerably more sensitive than this.

We describe here an analytical procedure for promethazine in serum in less than 5.0 µg/L concentrations, which would be suitable for establishing the bioequivalence of promethazine dosage forms after single-dose administration. Data illustrating its practicability for bioequivalence studies after oral and rectal administration of dosage forms of promethazine hydrochloride are presented.

Materials and Methods

Instrumentation

We used a Model 5020 high-performance liquid chromatograph equipped with an ultraviolet-visible detector and a Model 9176 recorder (both from Varian Associates, Palo Alto, CA 94303).

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For separations we used a 30 cm × 0.4 cm (i.d.) prepacked, reversed-phase column (MCH-10, Varian) and a mobile phase of methanol/distilled water (84/16 by vol), at a flow rate of 2.0 mL/min. The column effluent was monitored at 254 nm after sample extracts were injected onto the column by use of a 50-µL loop.

Reagents

Promethazine hydrochloride and trifluromazine were obtained from Wyeth Laboratories, Philadelphia, PA, and E. R. Squibb and Sons, Inc., Princeton, NJ, respectively. Pesticide-grade hexane was obtained from Fisher Scientific Co., Fair Lawn, NJ 07410; 2,2,2-trichloroethylchloroformate (TCECF) was from Aldrich Chemical Co., Inc., Milwaukee, WI 53200. Sodium carbonate was anhydrous and of the granular form.

The extracting solvent containing the internal standard was prepared by diluting 3.0 mL of a 1.0 mg/L methanolic solution of trifluromazine to 1000 mL with hexane. Plasma standards were prepared by placing 0.0, 0.25, 0.50, 1.0, and 2.0 mL of a 0.1 mg/L solution of promethazine in methanol into 16 × 150 mm Teflon-lined screw-cap culture tubes, evaporating the methanol, adding 10.0 mL of drug-free plasma, and vortex-mixing vigorously for 2 to 3 min. The resulting plasma-based standards containing 0.0, 2.5, 5.0, 10.0 and 20.0 µg/L were stable for several months if stored at −20 °C.

Procedure

Place exactly 2 mL of plasma or serum in a 16 × 150 mm screw-cap culture tube and buffer it with one Coors no. 01 porcelain spoon full of Na₂CO₃ (about 0.1 g) and vortex-mix for 3 s. Add 10 mL of hexane containing 3 µg of trifluromazine per liter to each sample as the extracting solvent. Extract by mixing the tube contents for 15 min on an automatic shaker, then centrifuge (2000 rpm, 5 min). Transfer about 9.3 mL of the hexane to a clean 16 × 125 mm screw-top culture tube and evaporate at 40 °C with a moderate air flow. Add 50 µL of ethyl acetate and 25 µL of trichloroethyl chloroformate (TCECF) to each tube. After vortex-mixing, incubate at 120 °C for 20 min, cool the derivatized samples, and evaporate the solvent with air at 40 °C. Dissolve residue in 100 µL of methanol and inject 50 µL of the solution directly into the liquid chromatograph.

Quantify by use of a standard curve in which the peak height ratio (promethazine/trifluromazine) is plotted vs promethazine concentration. Prepare standard curves by subjecting a series of plasma or serum samples containing known amounts of promethazine (0 – 20 µg/L) to the analytical procedure.

Results

Figure 1 shows typical chromatograms for the quantification of promethazine added to human plasma and in the sera of volunteers who had received promethazine hydrochloride (Phenergan, Wyeth) orally in a syrup formulation. Analysis of both standards and subject samples yielded as-yet-unidentified chromatographic peaks, which appeared early in the chromatograms but did not interfere with determination of promethazine. Furthermore, chromatograms I and IV
Patient Sera

Fig. 1. Liquid chromatograms of plasmas supplemented with promethazine (A) at concentrations of 0 μg/L (I), 5 μg/L (II), and 10 μg/L (III), and of patients' sera containing 0 (IV), 8.3 (V), and 35.6 μg (VI) of promethazine per liter. Triflupromazine (B), the internal standard, was added to each specimen to yield a concentration of 30 μg/L.

Table 1. Data for Promethazine Standard Curve

<table>
<thead>
<tr>
<th>Concentration (μg/L)</th>
<th>Promethazine Peak Height Ratio (SD)</th>
<th>Pt. Int. Ratio/ Promethazine Int. Ratio (SD)</th>
<th>SD, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0</td>
<td>0.0554 (0.002)</td>
<td>0.0554</td>
<td>14.3</td>
</tr>
<tr>
<td>2.5</td>
<td>0.1409 (0.010)</td>
<td>0.0564</td>
<td>9.6</td>
</tr>
<tr>
<td>5.0</td>
<td>0.2303 (0.016)</td>
<td>0.0461</td>
<td>7.1</td>
</tr>
<tr>
<td>10.0</td>
<td>0.4234 (0.034)</td>
<td>0.0423</td>
<td>7.1</td>
</tr>
<tr>
<td>20.0</td>
<td>0.9931 (0.071)</td>
<td>0.0497</td>
<td>7.2</td>
</tr>
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</table>

* Triflupromazine, internal standard.

b Relative SD (n = 5 at each concn.).

(Table 2), based on a sample signal equivalent to about 6.0% of the full-scale response (i.e., baseline noise approximates an apparent 0.5 μg/L). Recovery exceeded 90% for plasma specimens to which the drug was added.

Table 2 provides data on promethazine concentrations in serum after a 50-mg oral and rectal (suppository) dose of promethazine, administered successively to the same patient with a week intervening. About twice as much promethazine was present in the serum during the peak time of 180-240 min after oral as compared with rectal administration.

Several phenothiazine analogs—including chlorpromazine, chlorprothixene, phenothiazine-5-oxide, trimeprazine, promazine, triflupromazine—gave products after derivatization that chromatographed with retention times clearly different from that of the promethazine product. Only the undervarized phenothiazine nucleus gave a product with a retention time similar to that of promethazine. None of the phenothiazines that contained a piperazine group on the side chain reacted significantly with the TCECF reagent.

Discussion

Trichloroethyl chloroformate, a reagent that specifically derivatizes secondary and tertiary amine groups, was initially evaluated in our laboratory as a derivatizing reagent for determining promethazine at low nanogram per sample concentrations with use of gas chromatography with electron capture detection. Unfortunately, the reaction of promethazine with TCECF gives a product that is not as sensitive to this technique as are other phenothiazine analogs after their derivatization with the reagent. TCECF normally forms a carbamate with the tertiary amine groups to yield a product containing three chlorine atoms, making the corresponding products highly sensitive to the electron capture detector. Because the product from the reaction of TCECF with pro-
Table 2. Promethazine Concentrations in Serum after a 50-mg Oral or Rectal Dose (One Subject)

<table>
<thead>
<tr>
<th>Time, min</th>
<th>Oral concn, µg/L</th>
<th>Rectal concn, µg/L</th>
</tr>
</thead>
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<tr>
<td>0</td>
<td>&lt;1.0</td>
<td>&lt;1.0</td>
</tr>
<tr>
<td>20</td>
<td>2.0</td>
<td>1.1</td>
</tr>
<tr>
<td>40</td>
<td>3.1</td>
<td>2.9</td>
</tr>
<tr>
<td>60</td>
<td>3.8</td>
<td>5.3</td>
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<tr>
<td>90</td>
<td>9.3</td>
<td>6.8</td>
</tr>
<tr>
<td>120</td>
<td>21.6</td>
<td>8.7</td>
</tr>
<tr>
<td>180</td>
<td>23.1</td>
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</tr>
<tr>
<td>240</td>
<td>21.7</td>
<td>11.7</td>
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<tr>
<td>360</td>
<td>9.5</td>
<td>6.6</td>
</tr>
<tr>
<td>480</td>
<td>5.7</td>
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<tr>
<td>720</td>
<td>3.9</td>
<td>3.2</td>
</tr>
<tr>
<td>1440</td>
<td>&lt;1.0</td>
<td>1.1</td>
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</table>

Promethazine (and perhaps some of its metabolites) did not provide the required sensitivity by this technique, use of TCECF initially appeared impractical as an approach to the analysis of promethazine in biological extracts.

Subsequently, we investigated the feasibility of a chromatographic procedure involving the use of a fixed-wavelength (254 nm) ultraviolet detector. With derivatized promethazine, the detection limit in plasma was about 50 µg/L in biological extracts. Inadequate sensitivity resulted from normal serum constituents, which gave large interfering peaks near or at the point of promethazine elution. Back extraction from the hexane solvent was impractical as a way of removing the interfering endogenous substances from the extract, owing to instability of the drug in weakly acidic solutions.

We used several reagents in an attempt to make a derivative that could be assayed by liquid chromatography with ultraviolet detection. Although the TCECF derivative was only about 20% more sensitive to the ultraviolet detector than was the underivatized promethazine, the procedure either destroyed the interfering serum constituents or formed products with them that differed substantially in their elution characteristics from that of the promethazine derivative. This effect permitted operation of the ultraviolet detector at a much lower sensitivity than was possible before derivatization and resulted in a 50-fold increase in sensitivity for the analysis for promethazine in biological extracts. On a reversed-phase column (MCH-10) with a methanol/water system as the mobile phase, the absolute retention time for the promethazine derivative was about 4.2 min.

Many compounds were investigated for use as an internal standard, including several phenothiazine analogs. Triflupromazine gave a TCECF reaction product that eluted at a favorable time interval after promethazine, with good sensitivity and minimal interference from other drugs or endogenous serum constituents.

A comprehensive gas chromatographic/mass spectrometric analysis of the reaction product obtained from the reaction of promethazine with TCECF indicated the primary reaction product to be a phenothiazine analog that contained an isopropyl chloride group at position 10 (nitrogen) of the phenothiazine nucleus. In contrast to underivatized promethazine, this product was stable for two to three weeks in methanol. Other phenothiazine derivatives that reacted with TCECF, including the triflupromazine internal standard, gave the classic trichloroethyl carbamate derivative. Gas-chromatographic/mass spectrometric analysis also revealed that no sulfoxide metabolites were present in the promethazine product(s).

This method should be applicable to the determination of many phenothiazine compounds, particularly those that have a tertiary amine functional group containing at least one methyl group. Another application is for those phenothiazine analogs which lack halogen constituents in their molecular structures and are incapable of being assayed by gas chromatography with electron capture detection. For compounds that can be analyzed by this method, sensitivity and specificity are adequate for bioavailability studies and therapeutic drug monitoring.

The excellent technical assistance of John Ramirez is deeply appreciated. Susan Weintraub, Ph.D., performed GC/MS assays of the promethazine reaction product. This investigation was supported in part by Grant MD 26431-03 from the National Institute of Mental Health, NIH, Bethesda, MD, and in part by a grant from Alcon Laboratories, Inc., Fort Worth, TX.

References