of the three standard solutions were injected consecutively into the HPLC. With the aid of the data system, a three point linear calibration curve was constructed which plotted the weight (μg) of acetylcholine chloride injected as a function of area under the peak. The sample was quantitated by injecting 50 μL of the test solution and the weight (μg) of acetylcholine chloride obtained from the calibration curve. The test solution was prepared by transferring quantitatively the reconstituted solution into a 10.0-mL volumetric flask. The flask was brought to the mark with the mobile phase.

RESULTS AND DISCUSSION

Reverse-phase paired-ion HPLC was useful in separating intact acetylcholine from its decomposition products and its excipient, mannitol. Use of the mobile phase described gave a sharp, well resolved peak. The refractive index detector was the detector of choice since the drug lacks a chromophore; the sensitivity of the detector was more than adequate in quantitating acetylcholine chloride in the preparation.

Figure 1 illustrates the elution profile of acetylcholine. The retention time varied with the amount injected. The retention time increased with decreasing amounts of acetylcholine and ranged from 8.2 to 7.9 min for 50–150 μg of the acetylcholine standard. The variation in retention time is probably a manifestation of nonlinearity in the interaction of acetylcholine in the mobile phase with the stationary phase (reduction in interaction with increasing amounts of the drug). However, the variation of the retention time with the amount of acetylcholine injected did not negate the usefulness of the assay, since excellent linear curves were obtained. A standard curve constructed from 50, 100, and 150 g of the drug injected had a correlation coefficient of 0.9996.

Choline, acetic acid, and mannitol were shown not to interfere with the determination of acetylcholine (Fig. 2). One the the peaks in the sample displayed the same retention time, 5 min, as that of the authentic choline chloride solution. Acetic acid eluted with the solvent front and mannitol was retained on the column.

Three lots of the pharmaceutical preparation were analyzed by HPLC in duplicate and the results were compared with those from the hydroxylamine colorimetric method (2) (Table I). Precision data for the HPLC assay is presented in Table II. These results demonstrate the utility of the HPLC procedure; the method is simple, fast, specific, and stability indicating.

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Electron-Capture Capillary Gas Chromatographic Determination of Phenylpropanolamine in Human Plasma Following Derivatization with Trifluoroacetic Anhydride

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Abstract □ A capillary gas chromatographic analysis of phenylpropanolamine in human plasma, following extraction and derivatization with trifluoroacetic anhydride, is presented. Using an electron-capture detector, the method was sensitive enough to quantitate as little as 1 ng of drug/mL of plasma. The coefficient of variation from 5–262 ng/mL varied between 5.6 and 1.6%, respectively. Plasma concentration data following one 25-mg dose of phenylpropanolamine hydrochloride in four healthy volunteers illustrates the suitability of this analytical method for monitoring plasma levels after oral administration of a typical dosage form.

Keyphrases □ Capillary gas chromatography—phenylpropanolamine, human plasma, trifluoroacetic anhydride derivatization □ Phenylpropanolamine—capillary gas chromatography, human plasma, trifluoroacetic anhydride derivatization □ Derivatization—trifluoroacetic anhydride, capillary gas chromatography, human plasma

Previously reported methods for the determination of phenylalkanolamines and related compounds in biological fluids include gas chromatography (GC) after extraction and formation of perfluoroacyl or pentafluorobenzylimine-trimethylsilyl derivatives (1–10), GC after formation of a pentafluorophenyloxazolidine derivative (11), GC after extraction and detection with a nitrogen-selective detector (12), and HPLC following extraction and precolumn derivatization with *o*-phthalaldehyde (13), 4-chloro-7-nitrobenz-2,1,3-oxadiazole and sodium naphthaquinone-4-sulfonate (14), and phenyl isothiocyanate (15).

A procedure for extraction of phenylpropanolamine and an internal standard from plasma, derivatization with trifluoroacetic anhydride, separation by capillary GC, and detection by electron capture is presented in this report. The achievable detection limit of the method is 1 ng/mL. Plasma concentrations >5 ng/mL can be determined with accuracy and precision suitable for pharmacokinetic studies.

EXPERIMENTAL SECTION

Instrumentation—The gas chromatograph[1] was equipped with a capillary inlet system and an 8-mCi Ni^{63} electron-capture detector connected to an integrator-calculator[2]. The fused silica capillary column (0.25 mm i.d. × 30 m) was coated with polymethyl (5% phenyl) siloxane[3] to a final thickness of 0.25 μm.

The chromatographic conditions were as follows: injection volume of the

[1] 3700 Series gas chromatograph with a model 1070 capillary inlet system and pneumatics; Varian Instrument, Palo Alto, Calif.
[2] Model 4100; Spectra-Physics, Santa Clara, Calif.
[3] J & W Scientific, Rancho Cordova, Calif.

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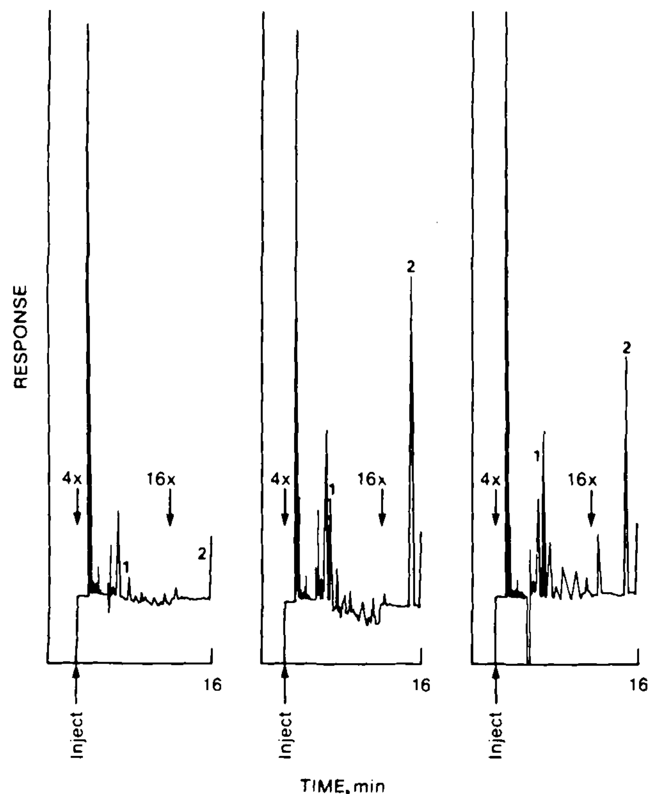

Figure 1—*Chromatogram of an analysis of a plasma blank (A), a plasma sample with added phenylpropanolamine and internal standard (5.2 and 200 ng/mL, respectively) (B), and a plasma sample from a subject dosed with 25 mg of phenylpropanolamine hydrochloride with added internal standard (18 and 200 ng/ml, respectively) (C). Key: (1) phenylpropanolamine (retention time = 10.8 min); (2) internal standard, 2-amino-3-phenyl-1-propanol, (retention time = 15.3 min). (Note: Chart speed was 0.1 cm/min until 9 min into each run, and 0.5 cm/min thereafter.)*

sample, 1-2 μL; injector temperature, 220°C; detector temperature, 250°C; column temperature, initially at 117°C for 10 min, programmed at 3°C/min to 125°C, followed by a 6-min hold at 125°C; helium (high purity) carrier gas flow, 0.3 mL/min; nitrogen (high purity) make-up gas flow, 30 mL/min. Injections were made in the split mode; the split ratio was 1:50.

Reagents—Phenylpropanolamine hydrochloride[4] and internal standard 2-amino-3-phenyl-1-propanol hydrochloride[5] were used to prepare standard solutions. 4-Dimethylaminopyridine[5], trifluoroacetic anhydride[6], and toluene[7] (UV grade, distilled in glass) were used as received. All other reagents were reagent grade.

Standard Solutions—The stock standard solutions (1 mg/mL) of phenylpropanolamine hydrochloride and 2-amino-3-phenyl-1-propanol hydrochloride were prepared in water and stored at 4°C.

Preparation and Extraction of Plasma Standards and Samples—Varying microliter volumes of a 0.001 dilution of phenylpropanolamine hydrochloride stock standard solution in water were added to 1-mL plasma aliquots to obtain concentrations of 5.2, 20.9, 104.7, 157.1, and 261.8 ng/mL of plasma. An aliquot (200 μL) of a 0.001 dilution of 2-amino-3-phenyl-1-propanol hydrochloride stock standard solution in water was added to each plasma standard and the sample (1 mL) for a final concentration of 200 ng/mL.

To the plasma standards and samples contained in 12-mL polytef-stoppered conical centrifuge tubes (silanized with 5% dimethyldichlorosilane[8] in toluene) were added 0.2 mL of 0.5 M KH_2PO_4 buffer (adjusted to pH 11.0 with NaOH) and 3 mL of toluene. The tubes were sealed and shaken gently for 30 min at room temperature on a horizontal shaker[9]. After centrifugation at 2000 rpm for 5 min, the organic layers were transferred to another set of 12-mL centrifuge tubes and concentrated to ~0.5 mL under nitrogen in a 40°C water bath. 4-Dimethylaminopyridine (0.2 mg dissolved in 50 μL of toluene, added as a catalyst) and trifluoroacetic anhydride (70 μL) were then added; the tubes were then stoppered and heated for 30 min in a 60°C water bath. Two milliliters of 0.5 M Na_2HPO_4 buffer (pH 6.0) was then added, followed by vortex-mixing for 15 s. Aliquots (1-2 μL) of the toluene layer were injected into the gas chromatograph.

[4] Sigma Chemical Co., St. Louis, Mo.
[5] Aldrich Chemical Co., Milwaukee, Wis.
[6] Pierce Chemical Co., Rockford, Ill.
[7] Burdick & Jackson Laboratory, Muskegon, Mich.
[8] Applied Science Division, Milton Roy Laboratory Group, State College, Pa.
[9] Eberbach Corp., Ann Arbor, Mich.

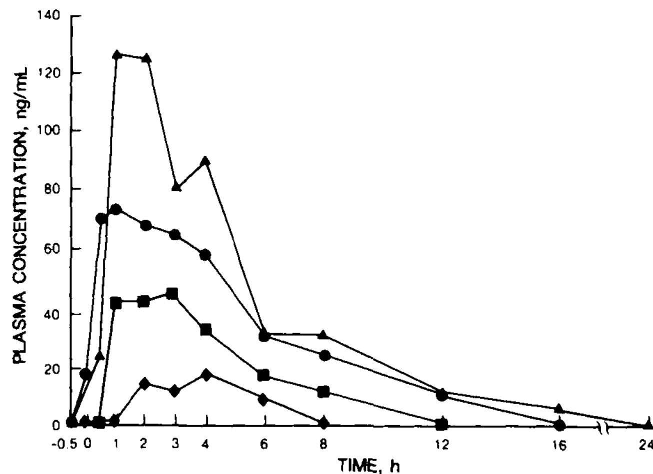

Figure 2—*Plasma levels of phenylpropanolamine base in four subjects following ingestion of 25 mg of phenylpropanolamine hydrochloride in water. Key: (●) subject 1; (▲) subject 2; (■) subject 3; (◆) subject 4.*

Standard Curve—The retention times of the di-trifluoroacetyl derivatives of phenylpropanolamine and 2-amino-3-phenyl-1-propanol were 10.8 and 15.3 min, respectively. The area responses for these chromatographic peaks were used to construct standard curves by plotting area ratio of phenylpropanolamine–internal standard against the respective phenylpropanolamine plasma concentration.

Clinical Study—As part of a clinical study, normal healthy subjects received a single 25-mg dose of phenylpropanolamine hydrochloride in water. Blood samples were collected at −0.5, 0, 0.5, 1, 2, 3, 4, 6, 8, 16, and 24 h in heparin-containing vacuum containers[10]. The plasma was separated immediately by centrifugation and frozen within 30 min. All samples were stored at −20°C until assayed.

RESULTS AND DISCUSSION

Figure 1 shows a typical chromatogram for phenylpropanolamine in spiked human plasma and in plasma obtained from a human subject after oral ingestion of 25 mg of phenylpropanolamine hydrochloride. Analysis of predose plasma samples from 24 human subjects presented no chromatographic peaks that interfered with either phenylpropanolamine or the internal standard. Plots of peak area ratios (phenylpropanolamine to internal standard) against phenylpropanolamine concentrations were linear. Typical standard curves for phenylpropanolamine in plasma had correlation coefficients of 0.9997.

The derivatization reaction was quantitative under the conditions described. This was determined in recovery experiments by monitoring the disappearance of underivatized drug in the reaction mixture. The reaction rate was increased several fold when 4-dimethylaminopyridine was present as a catalyst. The efficiency of extraction of drug from plasma was 60% due to the high water solubility of the phenylpropanolamine.

The coefficients of variation for five replicate assays at 5.2, 20.9, 104.7, 157.1, and 261.8 ng/mL of plasma were ±5.6, ±1.8, ±6.5, ±1.6, and ±1.6%, respectively. The lowest standard routinely used was 5 ng/mL, although the achievable detection limit of the assay was 1 ng/mL.

Figure 2, which shows typical plasma phenylpropanolamine levels following a single oral dose of 25 mg of phenylpropanolamine hydrochloride in water, demonstrates that the method is adequate for use in bioavailability studies.

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Determination of Sodium Levothyroxine in Bulk, Tablet, and Injection Formulations by High-Performance Liquid Chromatography

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Received July 15, 1983, from the *National Center for Drug Analysis, Food and Drug Administration, St. Louis, MO 63101.* Accepted for publication September 28, 1983.

Abstract □ Sodium levothyroxine was determined in bulk drugs, tablets, and injections by high-performance liquid chromatography (HPLC). Levothyroxine was separated from excipients and impurities on a 10-μm cyanoalkyl column using an acetonitrile-water-phosphoric acid mobile phase. The HPLC method is shown to be linear, accurate, and precise, and the results obtained by the HPLC and USP XX methods are compared.

Keyphrases □ Sodium levothyroxine—HPLC, determination of bulk, tablet, and injection formulations □ HPLC— sodium levothyroxine, determination of bulk, tablet, and injection formulations

In a survey[1] of sodium levothyroxine products and formulations, 63 samples of tablets, representing 20 formulations from 5 manufacturers, 9 samples of injections from 2 manufacturers, and 6 samples of bulk sodium levothyroxine from 5 manufacturers, were analyzed in this labroatory. The purpose of the survey was to evaluate the quality of sodium levothyroxine products on the market and the adequacy of present compendial standards and methods. Methodology was developed for content uniformity analysis of sodium levothyroxine by high-performance liquid chromatography (HPLC). This method, unlike the official compendial method (1), differentiates levothyroxine from iodinated impurities and degradation products.

Previously developed methods for the determination of sodium levothyroxine (2–13) were evaluated and tested. A modification of the HPLC procedure described by Garnick *et al.* (13), using a cyanoalkyl bonded phase column, was selected. This method offers advantages over those already in the literature by avoiding buffers in the mobile phase, no sample derivatization, faster analysis times, greater sensitivity due to shorter retention times, lower flow rates, and 229 nm detection. A sample solvent was selected that readily dissolved sodium levothyroxine without degradation from tablet formulations. This method and the results obtained on the survey sample are reported here.

[1] This study was a national survey for the Food and Drug Administration.

EXPERIMENTAL SECTION

Apparatus A modular high-performance liquid chromatograph[2] (HPLC) was equipped with a fixed-wavelength (229 nm) cadmium lamp UV detector[3], an automated injector[4], a microprocessor controller[5], and a recorder-integrator[6]. A stainless steel column (3.9 mm × 30 cm) was packed with irregular 10-μm silica particles to which a layer of cyanoalkyl silane was chemically bonded[7].

The mobile phase consisted of acetonitrile-water-phosphoric acid (350:650:1). This solution was passed through a 0.45-μm filter[8], deaerated, and then pumped through the HPLC system at a rate of 1 mL/min.

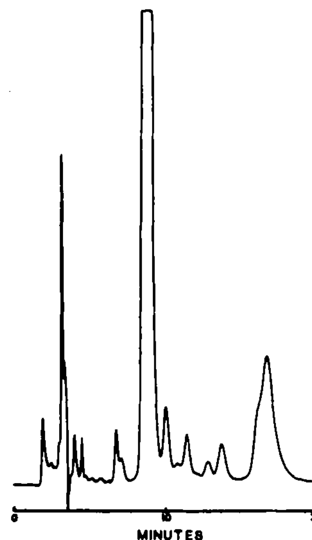

Figure 1—*Chromatogram of sodium levothyroxine bulk drug decomposed by heating in air; detector at 229 nm and 0.02 AUFS. Key: (1) sodium levothyroxine at a level of ~100 μg/mL.*

[2] Model ALC 204; Waters Associates.
[3] Model 441; Waters Associates.
[4] Model 710B WISP; Waters Associates.
[5] Model 720 System Controller; Waters Associates.
[6] Model 730 Data Module; Waters Associates.
[7] μ-Bondapak-CN; Waters Associates.
[8] Durapore UVLPO4700; Millipore Corp., Bedford, Mass.

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