Table II—Distribution of Carbon-14 in Organs 8 d after Peroral Administration of ^{14}C-Labeled Polymethyl Methacrylate Nanoparticles to Rats

	ng/g[a]
Blood	5.9 ± 1.6
Plasma	3.6 ± 1.1
Liver	12.7 ± 3.7
Spleen	5.1 ± 1.3
Pancreas	11.9 ± 4.6
Kidneys	9.6 ± 2.3
Adrenals	11.4 ± 6.2
Fatty renal tissue	24.5 ± 14.4
Testicles[b]	4.5
Uterus[c]	7.2
Epididymis[b]	9.0
Ovary[c]	7.0
Colon	5.0 ± 1.34
Stomach	14.5 ± 11.2
Small intestine	4.2 ± 0.6
Salivary glands	4.2 ± 1.4
Lymph nodes	13.3 ± 4.2
Thyroid glands	10.0 ± 4.8
Lungs	3.9 ± 1.4
Heart	4.1 ± 1.5
Muscles	2.6 ± 1.9
Bone marrow	66.5 ± 30.1
Skin	12.5 ± 7.1
Brain	4.3 ± 1.8
Stomach content	4.9 ± 2.3
Small intestine content	4.3 ± 1.6
Colon content	4.6 ± 2.7

[a] $n = 3$; mean, ±*SD*. [b] $n = 1$. [c] $n = 2$.

Since little radioactivity remained in the body after 8 d (Table II), the total absorption amounted to ~10–15% of the administered dose.

The absorption and excretion occurred rather rapidly. The biliary excretion rate reached a maximum after 1 h (Fig. 1) and then declined in a hyperbolic fashion. The maximal urinary excretion rate probably was reached within 2 h (Fig. 2) followed by a hyperbolic decline. Within 1 d ~83% and within 2 d ~95% of the total amount absorbed were eliminated from the body.

After 8 d, <0.5% of the administered dose remained in the body. The highest radioactivity (10 times more than in blood) was found in bone marrow. Higher levels of radioactivity were observed in renal fatty tissue, liver, pancreas, skin, adrenals, and the lymph nodes than in the blood. The values from the lymph nodes, however, give only an estimate of the actual value, due to the very small amounts of material available for the determination of the radioactivity in these organs.

The small amount of radioactivity absorbed from the GI tract probably consisted of low molecular weight components in the polymer. The figure of 10–15% is very similar to the 13% that was rapidly excreted after subcutaneous injection (6) and was, as mentioned above, attributed to low molecular weight components of the nanoparticle material. Whether these components can be used as carriers for nonabsorbable drugs is still an open question. The main part of the nanoparticle material, though capable of a carrier function, is not absorbable by rats. Because of the enhanced endocytotic activity of tumor cells (9), nanoparticles loaded with cytostatic carriers may be useful for the treatment of carcinomas of the GI tract.

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High-Performance Liquid Chromatographic Determination of Acetylcholine in a Pharmaceutical Preparation

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Received September 7, 1982, from the *Analytical Development Department, CooperVision Pharmaceutical, Inc., Mountain View, CA 94043.* Accepted for publication September 22, 1983.

Abstract □ A simple and rapid method for quantitating acetylcholine in a lyophilized preparation by high-performance liquid chromatography (HPLC) is described. A reverse-phase column with a refractive index detector was utilized for the assay. The HPLC system was able to separate acetylcholine from choline, a major degradation product, which was verified by running a degraded sample of a commercial preparation. The HPLC results were compared with the results obtained by a spectrophotometric procedure.

Keyphrases □ Acetylcholine—high-performance liquid chromatographic determination, lyophilized preparation □ High-performance liquid chromatography—determination of acetylcholine □ Lyophilized preparation—acetylcholine, high-performance liquid chromatographic determination

Acetylcholine is an endogenous parasympathomimetic agent thought to play an important role in the transmission of nerve impulses at synapses and myoneural junctions. Because the action of this parasympathetic stimulation is abrupt and fleeting, acetylcholine has limited therapeutic use. However, this unique property is presently being used to advantage in an intraocular irrigating fluid to obtain complete miosis instantaneously after delivery of the lens in cataract surgery. The product[1] is in a vial of two compartments: the lower chamber contains a lyophilized preparation of acetylcholine chloride with mannitol as a tonicity adjusting agent. The upper chamber contains sterile water for injection, and the two chambers are separated by a rubber plug. Since aqueous solutions of acetylcholine are unstable, the drug is reconstituted in sterile water just prior to use.

There have been several reports on chemical assays for acetylcholine. An excellent review of the current chemical methods has been published (1). The classical colorimetric method (2) suffers from lack of specificity.

Several gas chromatographic procedures have been reported. Primary means of detection have been either flame ionization (3) or mass spectrometry (4). In all cases, they involve volatilizing acetylcholine either by demethylation (5)

[1] Miochol, CooperVision, Inc.

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Table I—Comparison of Colorimetric and HPLC Methods for the Determination of Acetylcholine in Lyophilized Preparations

Lot	Acetylcholine Chloride, mg/vial	
	HPLC	Colorimetric
A	21.53	21.56
	21.22	
Mean	21.38	
B	21.90	20.67
	21.28	
Mean	21.59	
C	22.19	22.79
	21.84	
Mean	22.02	

or by pyrolysis in order to transform the molecule to the gas phase (6, 7).

A high-performance liquid chromatographic (HPLC) procedure (8) has been reported using a normal-phase column with phosphate buffered picrate ion as the stationary phase and with chloroform–butanol as the mobile phase. Separation of choline and acetylcholine has been achieved by cation-exchange chromatography (9).

This paper describes the application of a reverse-phase, paired-ion HPLC analysis of acetylcholine in the pharmaceutical preparation described above. The procedure is quick, specific, and stability indicating since it separates the drug from the principle degradation products, choline and acetic acid. A comparison of the results of HPLC and colorimetric methods was made.

EXPERIMENTAL SECTION

Chemicals and Reagents—Acetylcholine chloride[2], choline chloride[3], and mannitol[4] were used as received from the suppliers without further purification. Acetonitrile[5] was HPLC grade. Ammonium hydroxide[6] and acetic acid[6] were ACS grade or equivalent. The water used was glass distilled.

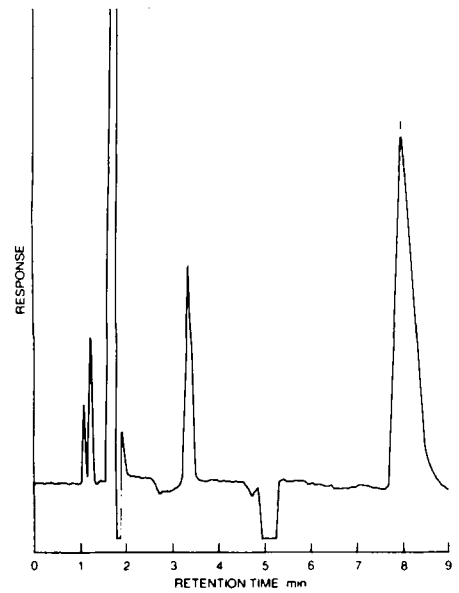

Figure 1—*Chromatogram of 50 μL injection of acetylcholine (I) standard (2 μg/μL).*

[2] Siegfried, Ltd.
[3] Eastman Kodak Co.
[4] J. T. Baker Chemical Co.
[5] Burdick & Jackson Labs., Inc.
[6] Fisher Scientific Co.

Table II—Precision Data for Acetylcholine in Lyophilized Preparations

Sample	Acetylcholine Chloride, mg/vial
1	21.62
2	21.22
3	22.04
4	21.23
5	21.70
6	20.96
Mean	21.46
SD	0.40
RSD, %	1.86

Instrumentation—The HPLC system consisted of a pump[7] capable of producing pressures of 6000 psi, a universal loop injector[8], a microparticulate reverse-phase column[9], a refractive index detector[10], and a microprocessor-controlled data system[11].

Mobile Phase—The mobile phase was prepared by adding sodium 1-heptanesulfonate[12] to 900 mL of water. After thorough mixing, 6 M ammonium hydroxide was added to adjust the pH to 4.0. After addition of 50 mL of acetonitrile, the solution was made up to 1 L with water.

Standard Stock Solution—Acetylcholine chloride, previously dried at 105°C for 4 h, was weighed (100 mg) into a 10.0-mL volumetric flask and dissolved in 5 mL of water. After mixing, the solution was brought to mark with water to make the stock standard solution.

Standard Solution—The standard solutions were prepared by pipetting 1.0, 2.0, and 3.0 mL of the stock standard solution to individual 10.0-mL volumetric flasks and bringing to mark with the mobile phase.

Decomposition of Acetylcholine—To prepare samples for heat stressing, the lyophilized solid in the commercial package was reconstituted in the sterile water. The solution was transferred quantitatively to a 10-mL clear glass ampule and the vial was washed with 1 mL of water; the wash solution was transferred to the ampule. After flame sealing, the ampule was placed in an oven at 130°C for 19 h. After cooling the ampule, the solution was transferred quantitatively to a 10.0-mL volumetric flask and was brought to the mark with the mobile phase.

Chromatographic Conditions—The volumetric flow rate was 2.0 mL/min, and the column temperature was ambient.

Standard Curve and Sample Analysis—Fifty-microliter volumes of each

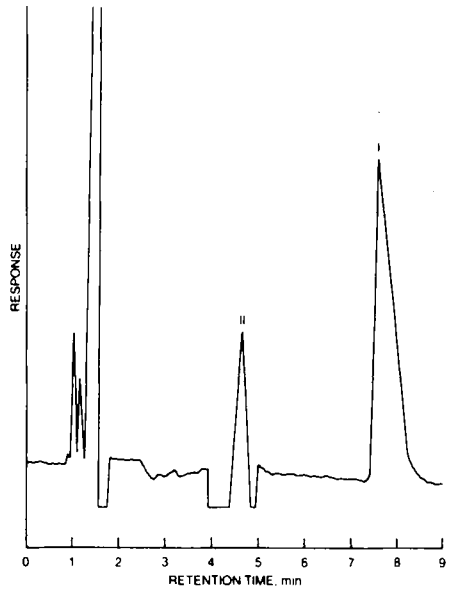

Figure 2—*Chromatogram of a heat-stressed sample of lyophilized preparation of acetylcholine chloride. The sample was heated at 130°C for 19 h. The final concentration of acetylcholine chloride was determined to be 11.13 mg/vial (55.65% of label claim). Key: (I) acetylcholine; (II) choline.*

[7] Model 6000A; Waters Associates.
[8] Model U6K; Waters Associates.
[9] μ-Bondapak C_{18}; Waters Associates.
[10] Model 401; Waters Associates.
[11] Model 4100; Spectra Physics.
[12] PIC Reagent B-7; Waters Associates.

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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