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ACKNOWLEDGMENTS

The authors are indebted to Ms. C. Johnston for assistance, to Dr. H. Dion for microbiological analysis, to Dr. L. M. Wheeler for encouragement and support, and to Ms. J. Pohl for assistance in preparing this manuscript.

GLC Determination of Trihexyphenidyl Hydrochloride Dosage Forms

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Received July 18, 1978, from the *U.S. Food and Drug Administration, Baltimore District, Baltimore, MD 21201.* Accepted for publication September 28, 1978.

Abstract □ A rapid, sensitive, and specific GLC method for the quantitation of trihexyphenidyl hydrochloride in various pharmaceutical dosage forms is described. The procedure involves chloroform extraction of the active ingredient from a weakly acidic solution, followed by GLC determination using a 3% methyl silicone column. The specificity of the system in relation to several compendial drug analogs also is reported.

Keyphrases □ Trihexyphenidyl hydrochloride—GLC analysis in various pharmaceutical dosage forms, analogs □ GLC—analysis, trihexyphenidyl hydrochloride, in various pharmaceutical dosage forms, analogs □ Antiparkinsonism agents—GLC analysis of trihexyphenidyl hydrochloride in various pharmaceutical dosage forms

Several methods for quantitating the antiparkinsonism drug trihexyphenidyl hydrochloride (I) in toxicological samples and dosage forms have been published. These procedures include nonaqueous titration (1–3), colorimetric determination (4–6), polarography (7), and fluorescence (8). The USP procedures for assaying I in elixirs (9) and tablets (10) consist of dye–complex formation with bromcresol purple, chloroform extraction, and colorimetric measurement. All of these analytical procedures are relatively nonspecific and may measure any similarly structured compounds present, leading to erroneous results. Although the more specific technique of GLC has been employed to quantitate I (11–17), little work has been reported on its use in assaying I in dosage forms. In addition, variable results have been encountered in this laboratory with the compendial methods.

The purpose of this work was to develop a rapid, sensitive, reproducible, and discriminating method for the determination of I in pharmaceutical formulations. The procedure presented involves the chloroform extraction of the active ingredient from weakly acidic sample solutions of elixirs, tablets, and sustained-release capsules, followed by GLC determination using a 3% methyl silicone column. The method is an adaption of the GLC procedure developed by Clark[1] for assaying phencyclidine hydrochloride in sample matrixes. This GLC system also permits the differentiation of I from several compendial analogs based on differing retention times.

[1] Charles C. Clark, U.S. Drug Enforcement Administration, Miami, Fla. Method and collaborative study results are currently in press.

EXPERIMENTAL

Reagents and Chemicals—All chemicals and reagents were USP, NF, ACS, or chromatographic grade.

Instrumentation—A pH meter[2] was fitted with a glass–calomel electrode system. The gas chromatograph[3] was equipped with a flame-ionization detector connected to an electronic integrator[4]. The glass column, 4 mm i.d. × 1.8 m long, was packed with 3% OV-1 on 100–120-mesh Chromosorb WHP[5] and conditioned at 260° for 24 hr under nitrogen at a flow rate of 30 ml/min. The instrument parameters were: injected quantity of sample solution, 4–5 μl; injector temperature, 240°; detector temperature, 240°; column temperature, 210° (isothermal); carrier gas (nitrogen) flow rate, 60 ml/min; hydrogen flow rate, 60 ml/min; and air flow rate, 240 ml/min, or according to manufacturer's recommendations.

Column temperature and flow rate were adjusted to elute I in about 7 min and the internal standard in about 8.8 min. (The relative retention time of I *versus* the internal standard is about 0.8.) Electrometer sensitivity was adjusted so that 4–6 μl of the standard solution gave a suitable recorder response, *i.e.*, 40–80% of full-scale deflection.

Preparation of Solutions—The dosage forms[6] studied were an elixir, 2 mg/5 ml; tablets, 2 mg; and sustained-release capsules, 5 mg. The stock solution of 1 mg of *n*-tricosane (II)/ml, the internal standard, was prepared by directly dissolving 100 mg of II in 100 ml of chloroform. The standard solution of I, 0.2 mg/ml, was prepared by treating 10 mg of the standard in the same way as the elixir but with 1% K_2HPO_4 for pH adjustment.

The standard mixture solution of I and its analogs, 1 mg/ml, was prepared by dissolving 10 mg each of cycrimine hydrochloride, biperidin hydrochloride, procyclidine hydrochloride, tridihexethyl chloride, and I in 10 ml of the internal standard solution and mixing.

Sample Preparation—*Elixir*—An amount of sample equivalent to 10 mg of I was pipetted into a 100-ml beaker. Volume was adjusted to about 50 ml with water. The pH was adjusted to 6 ± 0.5 by dropwise addition of 10% K_2HPO_4 (~1 ml) using a pH meter. The sample was then transferred quantitatively with water into a 125-ml separator.

Tablets—Not less than 20 tablets were weighed and finely ground. An accurately weighed portion of the powder, equivalent to about 10 mg of I, was transferred into a 125-ml separator. Twenty-five milliliters of hydrochloric acid (1 in 1000) was added, and the mixture was shaken vigorously mechanically for about 30 min. Then the contents were transferred to a 100-ml beaker, and the pH was adjusted to 6 ± 0.5 with dropwise addition of 1% K_2HPO_4 using a pH meter. The sample was transferred quantitatively with water back into the 125-ml separator.

Capsules (Sustained Release)—The contents of not less than 20 capsules were weighed and finely ground. An accurately weighed portion

[2] Orion model 701A.
[3] Hewlett-Packard model 5830A.
[4] Hewlett-Packard model 18850A.
[5] Applied Science Laboratories, State College, Pa.
[6] Artane, Lederle Laboratories, Pearl River, N.Y.

0022-3549/79/0400-0503$01.00/0

Table I—Assay Results [a] of I in Pharmaceutical Preparations following the USP, NDA, and GLC Methods

Dosage Form	USP/NDA	GLC
Elixir (2 mg/5 ml)	98.5	102.0
	105.2	103.5
	108.0	101.0
	102.5	98.9
SD	4.05	1.93
CV, %	3.91	1.90
Tablets (2 mg)	104.0	104.0
	103.6	105.5
	111.0	104.0
	107.5	104.5
	100.6	103.6
	103.7	
SD	3.64	0.73
CV, %	3.46	0.70
Sustained-release capsules (5 mg)	94.0	97.6
	93.4	96.4
	84.6	96.4
	94.2	95.2
	91.2	
	93.4	
SD	3.69	0.98
CV, %	4.02	1.02

[a] Shown in percent declared.

of the well-mixed powder, equivalent to about 10 mg of I, was transferred into a 125-ml separator.

Fifty milliliters of hydrochloric acid (1 in 1000) was added, and the mixture was shaken vigorously mechanically for about 30 min. Then the contents were transferred to a 100-ml beaker, and the pH was adjusted to 6 ± 0.5 by dropwise addition of 10% K_2HPO_4 (~1 ml) using a pH meter. The sample was transferred quantitatively with water back into the 125-ml separator.

Extraction—The standard, elixir, tablet, and capsule solutions were extracted with five 25-ml portions of chloroform. The extracts were filtered through a chloroform-washed cotton pledget into a 150-ml beaker. All extracts were evaporated carefully on a steam bath with a gentle current of air to about 25 ml. The residue was transferred quantitatively with chloroform to a 50-ml volumetric flask, to which had been added 10.0 ml of internal standard solution. The solution was diluted to volume with chloroform and mixed. Aliquots of 4–5 μl of the sample and standard solutions were injected into the gas chromatograph using a 10-μl syringe.

Table II—Recovery Results of I in Spiked Pharmaceutical Preparations with the GLC Method

Dosage Form	Added, mg	Found, mg	Recovery, %
Elixir	9.98	10.10	101.2
Tablets	9.98	10.15	101.7
Capsules	9.98	9.90	99.2

Calculations—The following formulas were used to calculate the I concentrations:

$$\text{mg/5 ml} = \left(\frac{A}{A'}\right)\left(\frac{I'}{I}\right)(CDF)\left(\frac{5}{S}\right) \qquad \text{(Eq. 1)}$$

$$\text{mg}\Big/\frac{\text{tablet or}}{\text{capsule}} = \left(\frac{A}{A'}\right)\left(\frac{I'}{I}\right)(CDF)\left(\frac{T}{W}\right) \qquad \text{(Eq. 2)}$$

where A and A' are the peak heights or areas of the sample and standard, respectively; I and I' are the peak heights or areas of the internal standard for the sample and standard, respectively; C is the concentration of standard (milligrams per milliliter); DF is the dilution factor; S is the sample aliquot (milliliters) taken; W is the weight of sample used (grams); and T is the average tablet or capsule net contents weight.

RESULTS AND DISCUSSION

Each dosage form studied was assayed by both the official USP (elixirs and tablets) or manufacturer's New Drug Application (NDA) (capsules) procedures and the proposed method. The results (Table I) show good correlation between the methods. The standard deviations and coefficients of variation obtained indicate that the proposed method is more precise than the USP and NDA methods for all forms analyzed.

Results of recovery studies for standard I added to each formulation are shown in Table II. The recoveries obtained were complete and consistent from product to product.

Gas chromatograms for the extracted standard and samples are presented in Fig. 1. Good peak symmetry, absence of interfering sample peaks, and short retention times for trihexyphenidyl (6.8 min) and II (8.8 min) were obtained. The GLC responses for trihexyphenidyl and II were linear over at least a range of 0.6–1.4 μg of drug injected. The minimum detectable level was 0.4 ng of trihexyphenidyl.

A study was conducted to determine the effect of pH on the extractability of the active ingredient in each type of formulation. A range between pH 5 and 7 was optimum for the total extraction of I in all dosage forms studied. Dibasic potassium phosphate or hydrochloric acid (1 in

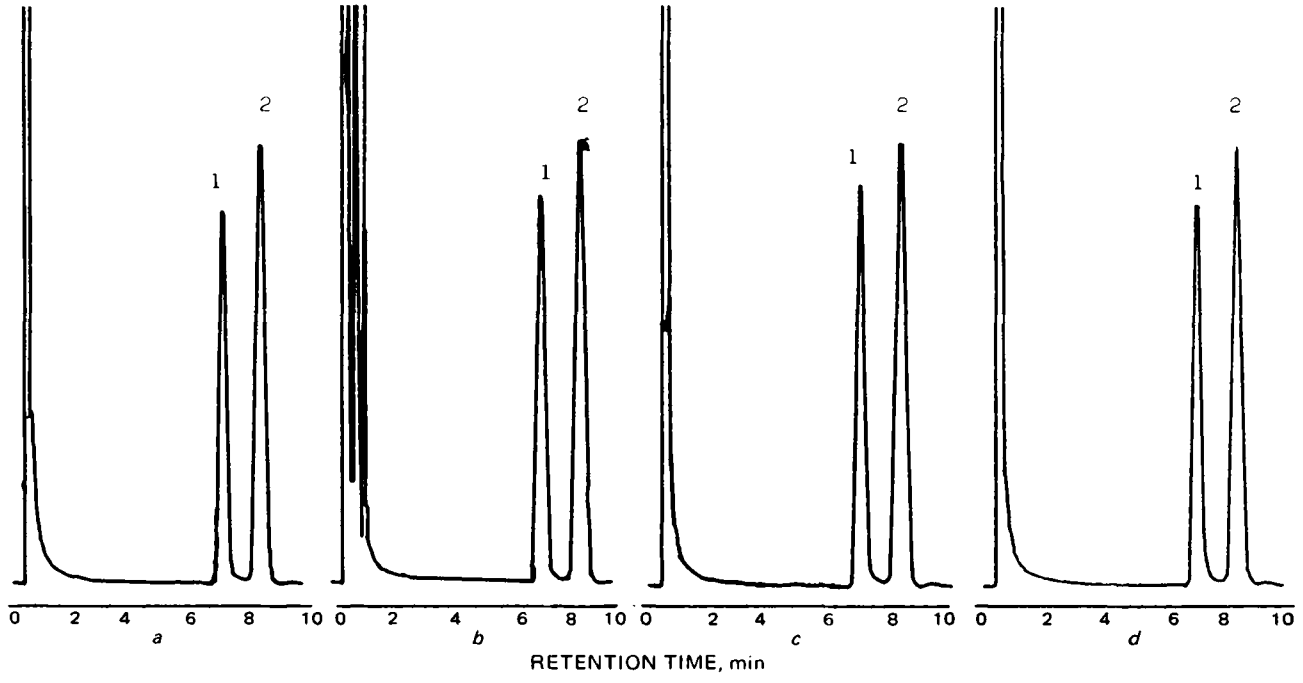
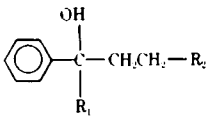
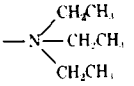


Figure 1—*Chromatograms of extracted solutions of:* (a) *standard I,* (b) *elixir (2 mg of I/5 ml),* (c) *tablets (2 mg of I), and* (d) *capsules (5 mg of I). Key: 1, trihexyphenidyl; and 2,* n-*tricosane.*

Table III—Chemical Structures and Relative GLC Retention Times of I and Compendial Drug Analogs



Compound	R_1	R_2	*RRT*[a]
Tridihexethyl			0.46
Cycrimine			0.56
Procyclidine			0.64
Trihexyphenidyl			0.80
Biperiden			0.88

[a] Relative retention time *versus* II, the internal standard.

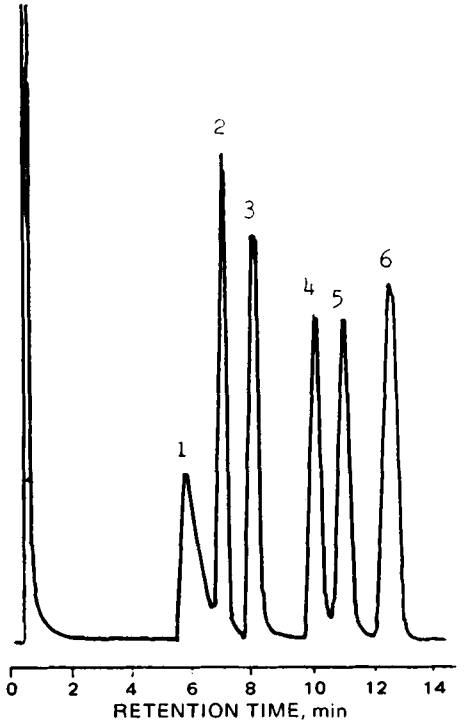


Figure 2—*Chromatogram of standard mixture of tridihexethyl chloride (1), cycrimine hydrochloride (2), procyclidine hydrochloride (3), I (4), biperiden hydrochloride (5), and II (6). Instrumental parameters and column conditions were as described under* Experimental, *except that the column temperature was 200°. All mixture components were at 1-mg/ml concentrations.*

1000) solutions were used to keep the sample and standard solutions within this pH range.

A literature search and discussions with various sources indicated that I is a stable compound under normal storage conditions. Little information was obtained on I degradation products, but the search for such reports continues. If degradation products are discovered, standards of such compounds will be obtained and their behavior in the proposed method will be tested. It is believed that chloroform-extractable degradation products would be detected by the proposed procedure, based on differing GLC retention times. Chromatograms obtained by the proposed method of 3-year-old elixir products stored at room temperature, of 3-month-old refrigerated I standard solutions, and of solutions from dissolution experiments involving blending of the capsule product for several minutes at high revolutions per minute in boiling water showed no extraneous peaks.

NF XIV contains monographs for procyclidine hydrochloride (18), tridihexethyl chloride (19), cycrimine hydrochloride (20), and biperiden hydrochloride (21), which are structurally related to I (Table III) and have similar pharmacological actions. The NF assays for these analogs all employ the bromcresol purple colorimetric procedure used in the USP assay for I. Thus, a study was undertaken to determine if these drugs could also be chromatographed successfully with the same GLC system developed for I. As indicated in Fig. 2, all compounds except tridihexethyl chloride exhibited excellent GLC peak shapes, short retention times (Table III), and near-baseline resolution from each other and from I and II.

This method may be used to differentiate I and its compendial analogs from one another and offers better specificity and precision than do the current compendial assays for I-containing formulations.

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ACKNOWLEDGMENTS

The author thanks Richard L. Everett and William M. Ment, U.S. Food and Drug Administration, Baltimore District, for assistance.