Determination of Cyclobenzaprine in Tablets by High-Performance Liquid Chromatography

MAXINE L. HEINITZ

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Abstract □ A convenient high-performance liquid chromatographic method for the quantitative determination of cyclobenzaprine hydrochloride in tablets is described. Samples were dissolved in 0.05 *N* sulfuric acid and diluted with methanol. The cyclobenzaprine hydrochloride was chromatographed using an octylsilane column and the eluent acetonitrile–0.6% dibasic potassium phosphate aqueous buffer (pH 3.0) (75:25) at a flow rate of 1.5 ml/min. Naphazoline hydrochloride was used as an internal standard. The UV detector response at 254 nm was linear for cyclobenzaprine hydrochloride in the 0.005–0.03 mg/ml range under conditions of the analysis.

Keyphrases □ Cyclobenzaprine hydrochloride—determination in tablets by high-performance liquid chromatography □ High-performance liquid chromatography—determination of amount of cyclobenzaprine in tablets □ Naphazoline hydrochloride—internal standard in high-performance liquid chromatographic test for cyclobenzaprine hydrochloride in tablets

The pharmacopeial determination of the skeletal muscle relaxant cyclobenzaprine hydrochloride is tedious and time consuming. The procedure (1) consists of extraction of the chloride ion pair into methylene chloride, followed by back extraction into dilute sulfuric acid and quantitative measurement by UV spectrophotometry. As any degradation product would be expected to exhibit UV absorbance, the USP procedure will not be indicative of stability.

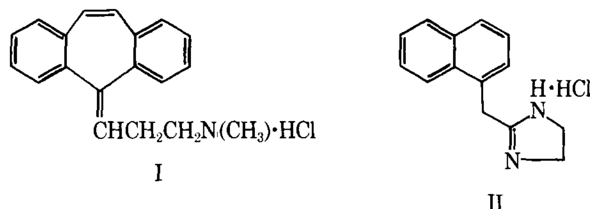
A number of procedures have been reported for the measurement of tricyclic antidepressant drugs in serum or in tablets by HPLC (2–11). Many of these methods use adsorption chromatography (2–6). Of those chromatographic systems utilizing reversed phases (7–11), the use of pentanesulfonic acid (10), sodium lauryl sulfate (11), or aliphatic amines (5) in the mobile phase was avoided. Premature deterioration of the column has been experienced in this laboratory when these reagents are used over an extended period of time. An eluent described previously (9) was adapted to the determination of cyclobenzaprine using an octyl-bonded silica column.

The procedure described was developed for the routine analysis of cyclobenzaprine hydrochloride in tablets. The method employs naphazoline hydrochloride as an internal standard and is readily adaptable to semi-automated analysis with equipment available in most laboratories.

EXPERIMENTAL

Chemicals—Cyclobenzaprine hydrochloride[1] raw material was assayed (100.5%) and its purity confirmed before use by the USP (1) monograph procedures. Naphazoline hydrochloride[2], amitriptyline hydrochloride[1], and desipramine hydrochloride[3] were used without further purification or analysis. All other reagents were commercial analytical reagent grade. Solvents[4] were glass distilled except for distilled water which was deionized and distilled.

Apparatus—The liquid chromatograph consisted of a pump and a



fixed wavelength UV detector[5] (254 nm). Solutions were injected by an automated sample processor[6] set to inject 20 μl. The chromatograms were recorded and results calculated by a reporting integrator[7]. UV spectra were recorded on a recording spectrophotometer[8].

Chromatographic Conditions—The mobile phase consisted of acetonitrile–0.6% KH_2PO_4 aqueous buffer (pH 3.0) (75:25). A flow rate of 1.50 ml/min was maintained. Ambient temperature was used throughout these experiments. A 25 × 0.46-cm i.d. column containing an octyl-bonded phase on silica packing[9] was used. Following each day of use, the column was flushed with acetonitrile–water (75:25), followed by methanol at 1 ml/min and stored in methanol.

Reagents—The pH 3.0 buffer was prepared by dissolving 12 g of potassium phosphate, monobasic in 1800 ml and adjusting to pH 3.0 with phosphoric acid (1:3) using a calibrated pH meter and diluting to 2 liters. Naphazoline hydrochloride prepared as a 1-mg/ml solution in methanol was present as the internal standard at a final concentration of 0.05 mg/ml in the sample solution. The standard solution contained 0.01 mg/ml of cyclobenzaprine hydrochloride and 0.05 mg/ml of naphazoline hydrochloride.

Assay and Calculations—Twenty tablets[10] were accurately weighed and finely powdered to pass a 60-mesh sieve. The equivalent of 1 tablet mass was transferred to a 100-ml volumetric flask, 25 ml of 0.05 *N* sulfuric acid in water was added and the flask was shaken for 1 hr. Methanol was added to a volume of ~85 ml, the flask contents were swirled, and allowed to equilibrate to room temperature. (The mixing of these solvents resulted in a warm solution.) The flask was diluted to volume and mixed. A 10.0-ml aliquot of each sample and 5.00 ml of a 1 mg/ml internal standard were diluted with water to 100 ml, and an aliquot was filtered[11]. For individual tablet analyses, each tablet was dissolved and diluted as described previously.

Duplicate 20-μl aliquots of composite solutions and single 20-μl aliquots of individual tablet solutions were chromatographed. Measurement was by peak area ratio of sample and reference standard to the internal standard using an average of two standard injections. Calculations were done by the reporting integrator.

RESULTS AND DISCUSSION

A typical chromatogram of cyclobenzaprine in the presence of naphazoline is shown in Fig. 1. Linearity of response was demonstrated by chromatographing standard solutions. The graph of response to variation in the concentration over the 0.005–0.030 mg/ml range of cyclobenzaprine hydrochloride with a constant naphazoline hydrochloride concentration (0.050 mg/ml) was linear and passed through the origin.

To determine the precision of this method as compared to the official USP assay, six replicate composites from each of four different commercial lots of cyclobenzaprine tablets were assayed. The results are tabulated in Table I. The coefficient of variation averaged 1.57% by HPLC and 1.00% by the USP method. The devised procedure was con-

[1] Merck Sharp and Dohme, West Point, Pa.
[2] CIBA Pharmaceutical Co., Summit, N.J.
[3] Merrell National Laboratories, Cincinnati, Ohio.
[4] Methanol and acetonitrile were from Burdick and Jackson Laboratories, Muskegon, Mich.; methylene chloride was from Mallinckrodt, Saint Louis, Mo.

[5] Altex Model 322, Altex Scientific, Berkeley, Calif.
[6] Waters Intelligent Sample Processor (WISP) Model 710A, Waters Associates, Milford, Mass.
[7] HP3385A, Hewlett-Packard, Avondale, Pa.
[8] Cary 118, Varian Associates, Palo Alto, Calif.
[9] Zorbax C-8, DuPont Instruments, Wilmington, Del.
[10] Flexeril tablets, Merck Sharp and Dohme, West Point, Pa.
[11] Metricel GA-6 (0.45μ), Gelman Instrument Co., Ann Arbor, Mich.

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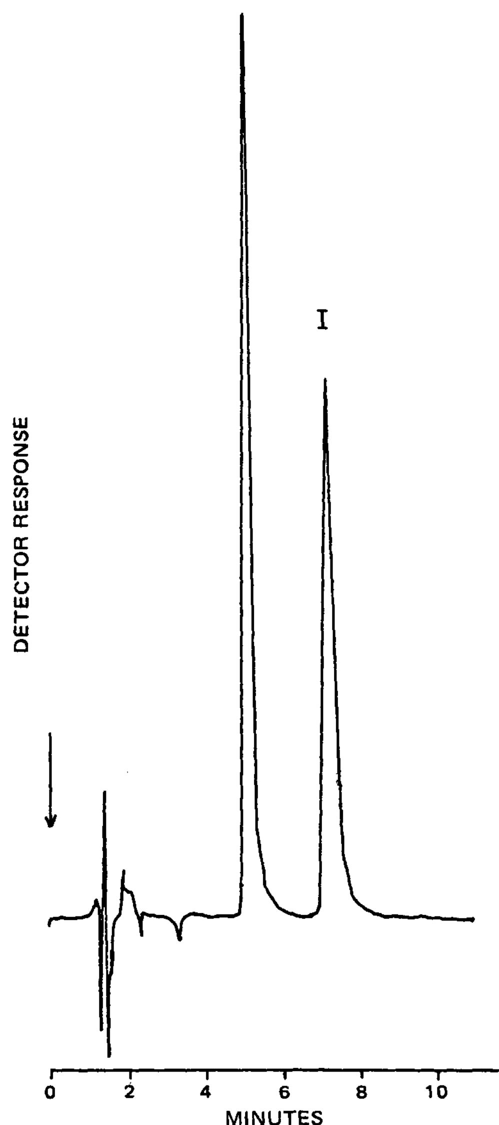

Figure 1—*Chromatogram of cyclobenzaprine hydrochloride tablet solution containing added naphazoline hydrochloride as internal standard. Key: (I) cyclobenzaprine; (II) naphazoline.*

sidered statistically acceptable. The results by liquid chromatography are slightly higher than those by the USP assay in three of four cases. Thirty tablets from each of the four lots were analyzed for content uniformity by the proposed HPLC method. The results are summarized in Table II.

During development of this assay a number of different variations of

Table I—Comparison of HPLC and USP Assay Results of Replicate Composite Preparations [a]

	Cyclobenzaprine Hydrochloride/Tablet, mg[b]			
	HPLC Method[c]		USP Method[d]	
	Mean	±*SD*	Mean	±*SD*
Sample 1	9.78	0.14	9.64	0.11
Sample 2	10.21	0.08	9.82	0.11
Sample 3	9.94	0.18	9.70	0.08
Sample 4	9.75	0.22	9.88	0.09

[a] Six replicate composites consisting of 20 tablets/composite were prepared from each of four commercial tablet lots. [b] Having a declaration of 10 mg of cyclobenzaprine hydrochloride/tablet. [c] Average of duplicate injections for each composite and standard solution. [d] The USP XX method for cyclobenzaprine hydrochloride tablets is extraction of the chloride ion pair into methylene chloride followed by back extraction into dilute sulfuric acid and quantitatively measured by UV spectrophotometry.

Table II—Results of 30 Individual Tablet Assays by HPLC [a]

Sample	Cyclobenzaprine Hydrochloride/Tablet, mg[b] Range	Mean	±*SD*	*RSD*, %
1	9.29–10.09	9.73	0.26	2.67
2	9.68–10.44	10.00	0.22	2.20
3	9.56–10.12	9.84	0.18	1.83
4	8.72–10.17	9.69	0.34	3.51

[a] Thirty tablets from each sample were assayed. [b] Declaration of 10 mg of cyclobenzaprine hydrochloride/tablet.

Table III—Relationship of the Retention Time to the Phosphate Content in the Aqueous Portion of the Mobile Phase [a]

	Retention Time, min		
% KH_2PO_4[b]	Cyclobenzaprine Hydrochloride	Amitriptyline Hydrochloride	Desipramine Hydrochloride
0.4	8.18	8.42	6.35
0.5	7.95	8.26	6.21
0.6	6.79	6.95	5.28

[a] The mobile phase consisted of acetonitrile–aqueous potassium phosphate pH 3.0 buffer (75:25); a 25 × 0.46-cm octyl-bonded phase on a silica column was used. [b] The phosphate content is expressed as the amount of monobasic potassium phosphate used to prepare the aqueous pH 3.0 buffer.

Table IV—Ultraviolet Absorption of Cyclobenzaprine Hydrochloride and Naphazoline Hydrochloride at Selected Wavelengths [a]

Compound	Wavelength, nm	$E^b \times 10^3$
Cyclobenzaprine hydrochloride	290	10.79 (max)
	254	10.26
Naphazoline hydrochloride	290	4.52 (sh)
	254	2.31

[a] Spectra recorded in water. [b] E = molar absorptivity.

the mobile phase were tried. The retention time of the cyclobenzaprine varied inversely with the acetonitrile content of the mobile phase. The proposed chromatographic system elutes cyclobenzaprine in ~7 min; but if the acetonitrile is reduced from 75:25 to 65:35, the retention time increases to 12 min. Substitution of methanol increases the retention time and magnifies the tailing.

The relationship of the phosphate content in the mobile phase to the retention time of cyclobenzaprine and two structurally related compounds is shown in Table III. As the concentration of phosphate decreases, the retention time increases.

Initially, only methanol was used to dissolve the tablet material. Choice of this solvent led to ~10% lower results by HPLC than by the USP procedure, even though measurement by UV spectrophotometry of the solution injected showed that the cyclobenzaprine was there at the expected level. Changing the solvent to water did not improve this disparity. When a small amount of acid was added to the solvent used to dissolve the tablet material, the HPLC results correlated well with those of the USP procedure. This problem may be caused by one of the tablet excipients. Examination of the chromatograms of solutions exhibiting low results did now show any evidence of band broadening. Some of the materials used in the tablet may be complexing with the cyclobenzaprine to either render the species immobile on the column, or in some other way interfere with the chromatography.

The use of a completely aqueous solvent for the tablet material was appealing because it would have eliminated the problem of heat formation on mixing the aqueous 0.05 N sulfuric acid with methanol. However, an entirely aqueous solvent, or acetonitrile and water, carry into solution most of the tablet excipients, which would then be injected on the column. By the use of methanol, many of the excipients can be removed by filtration prior to chromatography.

Naphazoline hydrochloride was selected for the internal standard for this study because it produced a symmetrical peak shape with good retention characteristics. The molecule possesses a large π-electron system with a good UV chromaphore.

The detector wavelength used throughout this study was 254 nm. More specificity would be gained by the use of the detector wavelength at 290 nm. The UV spectral parameters of cyclobenzaprine and naphazoline at these wavelengths are tabulated in Table IV. At 290 nm half the amount of naphazoline hydrochloride would give a detector response comparable to the response observed at 254 nm.

The HPLC assay procedure is rapid, precise, and specific for the analysis of cyclobenzaprine hydrochloride in tablets and represents a more convenient assay method than that of the USP.

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Derivatization of Chiral Amines with (*S*,*S*)-*N*-Trifluoroacetylproline Anhydride for GC Estimation of Enantiomeric Composition

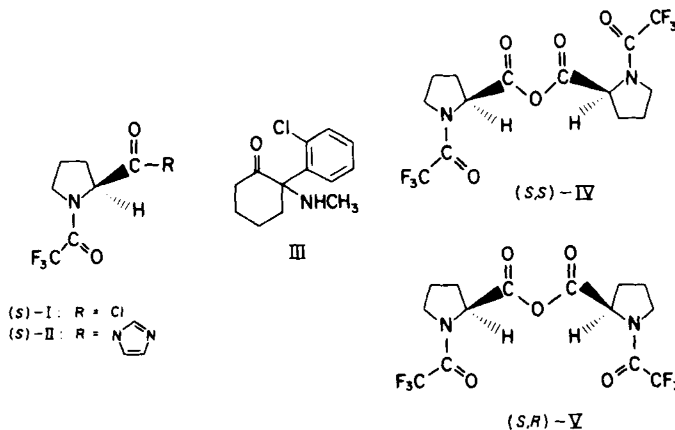
JAMES D. ADAMS, Jr. *§x, **THOMAS F. WOOLF** ‡, **ANTHONY J. TREVOR** *, **LYALL R. WILLIAMS** ‡, and **NEAL CASTAGNOLI, Jr.** ‡

Received June 8, 1981, from the * *Department of Pharmacology and the* ‡ *Department of Pharmaceutical Chemistry, University of California, San Francisco, CA 94143.* Accepted for publication September 16, 1981. § Present address: Department of Internal Medicine, Baylor College of Medicine, Texas Medical Center, Houston, TX 77030.

Abstract □ The reaction characteristics of (*S*,*S*)-*N*-trifluoroacetylproline anhydride were examined in an attempt to develop a quantitative GC assay of the enantiomers of the sterically hindered, chiral amine ketamine. With the aid of the individual enantiomers of ketamine and the corresponding synthetic *N*-trifluoroacetylprolyl amides, it was found that the derivatization reaction proceeds stereoselectively, in poor yield, and with some degree of racemization of the acylating reagent. The results indicate that care must be exercised when prolyl derivatizing reagents are chosen for assaying chiral amines.

Keyphrases □ (*S*,*S*)-*N*-Trifluoroacetylproline anhydride—derivatization of ketamine, GC estimation of enantiomeric composition □ GC analysis—detection of enantiomers of chiral amines with (*S*,*S*)-*N*-trifluoroacetylproline anhydride □ Enantiomers—derivatization of chiral amines with (*S*,*S*)-*N*-trifluoroacetylproline anhydride for GC estimation of enantiomeric composition

It is important to examine the extent to which chiral xenobiotics may undergo enantioselective metabolic transformations in an effort to characterize the effects which such processes may have on the pharmacological and toxicological properties of these substances (1, 2). In the case of chiral amines, quantitative estimations of enantiomeric composition have been achieved through GC analysis of the diastereomeric amides formed by derivatization with (*S*)-*N*-trifluoroacetylprolyl chloride [(*S*)-I] (3). Although commercially available as a solution in chloroform, this reagent is difficult to obtain in pure form and is susceptible to racemization (4). The corresponding imidazolide, compound (*S*)-II, is a relatively stable, crystalline solid (5). However, it was observed that this derivative reacts sluggishly with sterically hindered amines such as ketamine (III) (6). In an attempt to obtain a derivatizing reagent that can be prepared in crystalline form and that might react more readily with ketamine, the synthesis of (*S*,*S*)-*N*-trifluoroacetylproline anhydride [(*S*,*S*)-IV] was attempted. An attractive feature of (*S*,*S*)-IV is that inversion about one of the two chiral centers present in this molecule leads to the *meso*-diastereomeric species (*S*,*R*)-V which, in theory, should be separable from (*S*,*S*)-IV.



RESULTS AND DISCUSSION

The synthesis of (*S*,*S*)-IV from (*S*)-proline and trifluoroacetic anhydride was reported originally by Weygand (7). Attempts to repeat this synthesis initially led to the isolation of a species (mp 114–115°) which proved to be an isomer of the Weygand compound; longer reaction times however provided the Weygand compound (mp 138–140°). The electron impact mass spectra and NMR spectra of these products were essentially identical, which suggested that the two compounds were diastereomerically related. Since the high-melting isomer did not rotate plane polarized light, whereas the low-melting isomer was strongly levorotatory, the low-melting isomer was tentatively assigned the asymmetric structure (*S*,*S*)-IV and the Weygand compound the *meso*-structure, (*S*,*R*)-V.

Consistent with these assignments, reaction of (*S*,*S*)-IV with (*S*)-

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