GLC Determination of Cyclobenzaprine in Plasma and Urine

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Abstract □ A GLC determination of cyclobenzaprine in human plasma and urine is described. After extraction from alkalinized samples with heptane–isopentyl alcohol (97:3), the drug and internal standard were back-extracted into 0.1 *N* HCl and then reextracted into ether. Use of a lower homolog of the drug as an internal standard was effective in reducing variability. Drug concentrations as low as 25 ng/ml could be assayed with high precision. Plasma levels in humans given 40 mg po or iv ranged from 5 to 51 ng/ml; little unchanged cyclobenzaprine was present in the urine. The *N*-desmethyl analog of the drug was detected as a metabolite in urine.

Keyphrases □ Cyclobenzaprine—GLC analysis, plasma and urine □ GLC—analysis, cyclobenzaprine, plasma and urine □ Sedatives—cyclobenzaprine, GLC analysis, plasma and urine

Cyclobenzaprine, *N,N*-dimethyl-5*H*-dibenzo[*a,d*]-cycloheptene-Δ^5,γ-propylamine, is a unique, centrally acting, skeletal muscle relaxant currently in clinical trial (1, 2). Since knowledge of plasma concentrations is often of value in adjusting dosage and dosage schedules to obtain optimal therapeutic response, a GLC method for the drug in plasma was developed. The procedure used is similar to that previously described for the analysis of amitriptyline (3). The method is also applicable to the analysis of the drug in urine and may be an attractive alternative in this case to a previously proposed assay (4), based on quantitative TLC, which requires careful control of several parameters.

EXPERIMENTAL

Reagents—*n*-Heptane[1] was used without further purification. Isopentyl alcohol[2] was washed successively with 1 *N* NaOH, 1 *N* HCl, and water before use. Ether[3] was redistilled immediately before use, and ethyl acetate[4] was redistilled before use. Double-distilled water was used to prepare 0.5 *N* NaOH (from the solid reagent[5]) and 0.1 *N* HCl (by dilution of the concentrated acid[6]).

Cyclobenzaprine and desmethylcyclobenzaprine were used as their hydrochloride salts[7]; the internal standard, *N,N*-dimethyl-5*H*-dibenzo[*a,d*]cycloheptene-Δ^5,γ-ethylamine, was used as the hydrogen maleate salt[7]. Concentrations of each drug are expressed as the free base. Solutions of drug and internal standard were prepared in double-distilled water.

Apparatus—A gas chromatograph[8] with a hydrogen flame-ionization detector was used. A glass column, 1.8 × 4 mm i.d., packed with 1.5% OV-17 on 80–100-mesh Gas Chrom Q[9], was conditioned before use by heating at 250° overnight under helium flow. Analyses were performed under the following conditions: injection port temperature, 260°; column temperature, 218°; and detector temperature, 285°. The gas flows were: hydrogen, 50 ml/min; air, 450 ml/min; and helium carrier gas, 100 ml/min. The recorder was used on range 1 (4×10^{-12} amp) and attenuation 16.

Disposable glass tubes with constricted tips[10] were used for evaporation of the final ether extract and were cleaned by washing with detergent, rinsing thoroughly with double-distilled water, draining, and drying in a vacuum oven at 100°.

Procedure—Two milliliters of plasma or urine, 200 ng of internal standard (in 0.1 ml of water), 1 ml of 0.5 *N* NaOH, and 25 ml of *n*-heptane–isopentyl alcohol (97:3) were shaken for 20 min in a 45-ml glass-stoppered centrifuge tube. The tube was centrifuged, and as much of the organic phase as possible was transferred to a similar tube containing 5 ml of 0.1 *N* HCl. The tube was shaken for 10 min and centrifuged, and the organic (upper) phase was discarded by aspiration.

The aqueous phase was washed three times with *n*-heptane (25 ml each) to remove any residual isopentyl alcohol, the washings being discarded each time by aspiration. Then the aqueous phase was transferred to a clean 13-ml glass-stoppered centrifuge tube. Five milliliters of freshly distilled ether and 1.5 ml of 0.5 *N* NaOH were added, and the tube was shaken for 10 min. After centrifuging, the ether layer was transferred with a Pasteur pipet to a glass tube with a constricted tip.

The solvent was removed in a warm water bath (40°), with periodic chilling in ice water to rinse down the sides. The residue was dissolved in 25 μl of ethyl acetate, and a 5-μl aliquot was injected into the chromatograph. All samples were assayed in duplicate.

Following chromatography, a baseline was drawn and peak heights of cyclobenzaprine and the internal standard were measured. The ratio of cyclobenzaprine and internal standard peak heights was calculated, and the concentration of drug was obtained by reference to the standard curve.

A standard curve was constructed by analysis of samples to which known amounts of cyclobenzaprine and internal standard had been added. After plotting the peak height ratios of drug to internal standard *versus* cyclobenzaprine concentration, the best-fit straight line was drawn that passed through the experimental points and the origin.

RESULTS AND DISCUSSION

Cyclobenzaprine and the internal standard were adequately separated under these conditions (Fig. 1) with retention times of 2.8 and 2.2 min, respectively. Possible metabolites of the drug, the mono- and didesmethyl analogs, had retention times of 3.2 and 3.1 min, respectively, and thus would not interfere in the analysis. Further evidence of the specificity of the method may be inferred from the fact that the drug was well separated from the internal standard, which, in this case, was the closely related homologous compound containing a two-, rather than three-, carbon side chain.

The precision and accuracy of the method are demonstrated in Tables I and II. The mean relative standard deviations were 9.0% for plasma and 6.7% for urine[11]. The limit of detection of the method was approximately 4 ng/ml of plasma, but the practical limit of sensitivity was about 10 ng/ml. Sensitivity could be increased by using larger plasma volumes since plasma extracts showed negligible interfering peaks.

The plasma levels and urinary excretion of cyclobenzaprine in two

[1] Pesticide grade, Matheson, Coleman and Bell, East Rutherford, N.J.
[2] Reagent grade, J. T. Baker Co., Phillipsburg, N.J.
[3] Anhydrous, analytical reagent ACS grade, Fisher Scientific Co., King of Prussia, Pa.
[4] Fisher certified ACS grade.
[5] Fisher certified grade.
[6] Analytical reagent grade, J. T. Baker Co.
[7] Synthesized in the Merck Sharp and Dohme Research Laboratories.
[8] Hewlett-Packard model 810 equipped with a model 7123A recorder.
[9] Applied Science Laboratories, State College, Pa.
[10] Laboratory Research Co., Los Angeles, Calif.

[11] The following formula was used to estimate the mean relative standard deviation:

$$\text{mean } RSD = \frac{\text{pooled } SD}{\text{overall average}}$$

where pooled *SD* (*Sp*) is estimated as

$$Sp = \left[\frac{(n_1 - 1)S_1^2 + \ldots + (n_k - 1)S_k^2}{(n_1 - 1) + \ldots + (n_k - 1)}\right]^{1/2}$$

and:

$$\text{overall average} = \frac{n_1\bar{X}_1 + \ldots + n_k\bar{X}_k}{n_1 + \ldots + n_k}$$

for k groups with $n_1, n_2 \ldots, n_k$ observations/group.

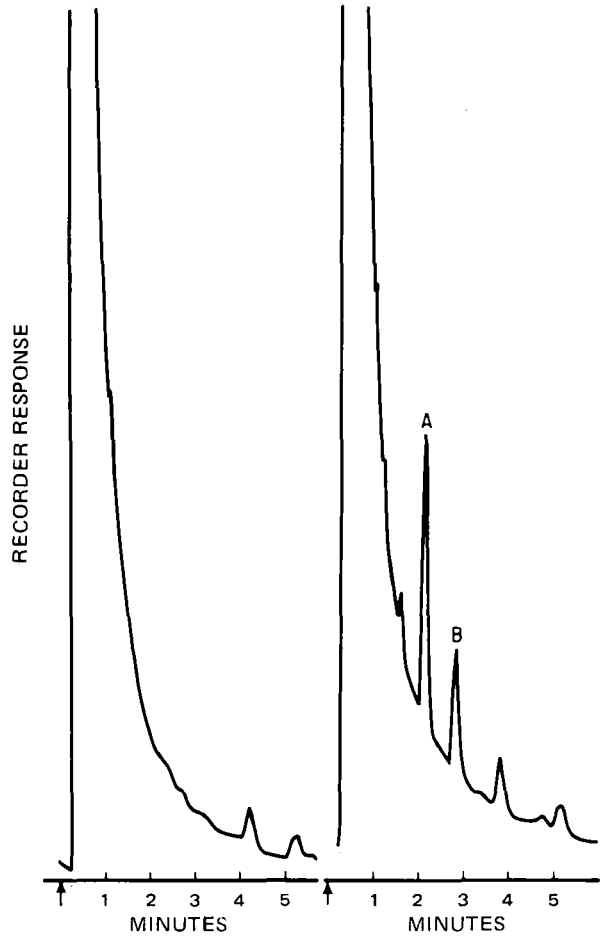

Figure 1—*Gas chromatogram of an extract of 2 ml of control human plasma containing no drugs (left) and of an extract of 2 ml of human plasma containing 50 ng/ml of cyclobenzaprine and 100 ng/ml of internal standard (right). Key: arrow, time of injection; A, internal standard peak; and B, cyclobenzaprine peak.*

human subjects after oral and intravenous administration of a 40-mg dose are shown in Tables III and IV. These preliminary results suggested that the drug is somewhat slowly absorbed, since peak plasma levels of 29.6 ng/ml were observed after 4–6 hr. Intravenous administration of the drug gave plasma levels that were somewhat unusual in that the sample taken after 15 min was no higher than the samples taken over the following 2 hr.

Table I—Accuracy and Precision of GLC Analysis of Cyclobenzaprine Added to Human Plasma[a]

Added, ng/ml	Found, Mean (Range)	*SD*	*RSD*, %
250	228 (210–262)	16.2	7.1
100	100 (92–105)	7.0	7.0
50	50 (46–54)	2.8	5.6
25	24 (22–30)	3.2	13.1

[a] Number of analyses was eight in all cases.

Table II—Accuracy and Precision of GLC Analysis of Cyclobenzaprine Added to Human Urine[a]

Added, ng/ml	Found, Mean (Range)	*SD*	*RSD*, %
250	244 (228–258)	12.5	5.1
100	99 (95–105)	5.1	5.1
50	47 (45–50)	2.2	4.6
25	24 (20–25)	2.4	10.2

[a] Number of analyses was four in all cases.

Table III—Plasma Levels of Cyclobenzaprine in Human Subjects after Oral and Intravenous Doses[a]

Hours	Drug Concentration, ng/ml	
	Oral Dose	Intravenous Dose
0.25	6.4 ± 3.1	48.2 ± 11.7
0.5	10.5 ± 2.1	51.1 ± 9.2
0.75	5.1 ± 1.6	46.7 ± 3.8
1	11.6 ± 9.2	45.0 ± 0.3
2	15.4 ± 8.0	51.0 ± 19.2
3	17.6 ± 1.7	38.8 ± 2.7
4	28.8 ± 0.4	43.0 ± 12.8
6	29.6 ± 13.1	39.4 ± 6.7
8	24.3 ± 6.9	26.6 ± 0.6

[a] The subjects received a 40-mg single dose of the drug by one route followed 7 days later with the same dose by the second route. The figures represent the mean ± *SD* of two subjects.

Table IV—Urinary Excretion of Cyclobenzaprine in Human Subjects after Oral and Intravenous Doses[a]

Hours	Cyclobenzaprine in Urine	
	Oral Dose	Intravenous Dose
0–2	0.01 ± 0.00	0.21 ± 0.05
2–3	0.03 ± 0.01	0.27 ± 0.35
3–4	0.02 ± 0.01	0.09 ± 0.10
4–8	0.04 ± 0.01	0.28 ± 0.21
8–24	0.10 ± 0.01	0.67 ± 0.78
Total	0.20 ± 0.01	1.52 ± 1.39

[a] Dosage was as described for the same two subjects in Table III. Results are expressed as the mean percent of dose excreted ± *SD*.

This finding suggests, as does an estimate of the apparent volume of distribution, that cyclobenzaprine is rapidly taken up by tissues from which sites the drug is slowly and relatively constantly released into the blood. Levels after the intravenous dose were considerably

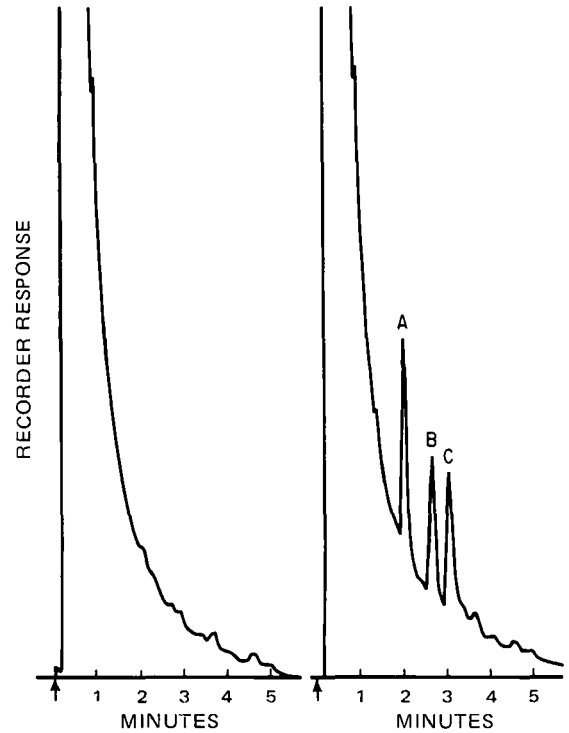

Figure 2—*Gas chromatogram of an extract of 2 ml of control human urine containing no drugs (left) and of an extract of 2 ml of human urine collected 8–24 hr after oral administration of 40 mg of cyclobenzaprine (right). Key: arrow, time of injection; A, internal standard peak; B, cyclobenzaprine peak; and C, desmethylcyclobenzaprine peak.*

higher during the first 4 hr than those attained following the oral dose, suggesting also that first-pass hepatic metabolism is important and that this phenomenon may be involved in the pharmacokinetics of cyclobenzaprine absorption in humans.

The presence of only a relatively small amount of unchanged drug in the urine (0.2–1.5% of the administered doses) would suggest that the drug was virtually completely metabolized in humans. The extent of metabolism may not be quite this complete since, after administration of ^{14}C-labeled drug[12], only 6.3% of a 10-mg po dose and 7.1% of a 10-mg iv dose were excreted in the 24-hr urine.

No peaks attributable to metabolites of cyclobenzaprine in plasma were observed during assay of the human samples. Norcyclobenzaprine was presumably present in the plasma, since it was found in the urine, but the detection limit for the desmethyl derivative was much higher than for cyclobenzaprine because of less favorable GLC and extraction properties[12]. In contrast, nortriptyline is readily detected in plasma after administration of amitriptyline, a drug having a structure very similar to that of cyclobenzaprine (3, 5, 6).

GLC analysis of the urine samples revealed the presence of an additional peak at 3.2 min, the same retention time as that of *N*-desmethylcyclobenzaprine (Fig. 2). Treatment of the final ethyl acetate extract with trifluoroacetic anhydride before GLC analysis caused the disappearance of the 3.2-min peak and the appearance of a new peak at 5.0 min, the same retention time as that of the trifluoroacetyl derivative of authentic *N*-desmethylcyclobenzaprine. The possibility that the metabolite was didesmethylcyclobenzaprine was ruled out by the fact that, although the retention time of the free amine was similar to that of the metabolite, the retention time of its trifluoroacetyl derivative was 4.0 min.

The desmethyl metabolite was most predominant in the 8–24-hr urine samples and appeared to be present in equal or higher concentrations compared to unchanged cyclobenzaprine. Evidently, the *N*-desmethyl metabolite was present in plasma in concentrations too low to be detected, but urinary levels were sufficiently high for identification.

The present GLC method is suitable for the quantitative assay of cyclobenzaprine in human plasma and urine samples after administration of a single 40-mg dose. Greater sensitivity may be necessary when a smaller dose is administered and can be achieved by using a larger plasma sample for assay.

[12] Unpublished work.

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Kinetics of Acid Neutralization by Aluminum Hydroxide Gel

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Abstract □ The rate of acid neutralization by an aluminum hydroxide gel prepared by the reaction of aluminum chloride solution and strong ammonia solution was studied. The decrease in acid-consuming capacity during aging as measured by the USP test is due to a decrease in the rate of reaction rather than to a decrease in equilibrium reactivity. The reactivity profile has three phases, which are shown to be related to the structure of the gel. The rate of loss of reactivity is directly related to the extent of washing.

Keyphrases □ Aluminum hydroxide gel—kinetics of acid neutralization, effect of aging, reactivity related to gel structure □ Acid neutralization—by aluminum hydroxide gel, effect of aging, reactivity related to gel structure □ Gels—aluminum hydroxide, kinetics of acid neutralization, reactivity related to gel structure □ Antacids—aluminum hydroxide gel, kinetics of acid neutralization, effect of aging

Aluminum hydroxide gel is known to lose acid reactivity on aging (1–3). The purposes of this investigation were: (*a*) to study this neutralization reaction for an aluminum hydroxide gel prepared by the reaction of aluminum chloride solution with strong ammonia solution; and (*b*) to determine whether the decrease in acid reactivity, as determined by a modification of the USP acid-consuming capacity test (4), was attributable to a decreased rate of reaction or to a decrease in equilibrium reactivity.

EXPERIMENTAL

Materials—All chemicals used were either reagent or analytical grade.

Aluminum Hydroxide Gel Preparation—A 4-liter batch of gel was prepared by the addition of 13% (v/v) strong ammonia solution to a solution of 287.2 g of aluminum chloride hexahydrate in 3340 ml of distilled water. Strong ammonia solution was added with agitation at a rate of approximately 120 ml/min to a final pH of 7.0. After precipitation, the gel was divided into four equal portions. The first portion (I) was washed with 1 liter of distilled water by draining through a canvas bag, and a second portion (II) was washed with 5 liters of distilled water. The gels were diluted to 1 liter with distilled water, stored in tightly closed glass containers, and aged at 25°.

Analytical Procedures—The equivalent aluminum oxide content was determined by the ethylenediaminetetraacetic acid titration (4). The gels contained between 1.40 and 1.50% equivalent of aluminum oxide.

The total chloride content was determined by the Volhard titration (5) after 3 g of gel was dissolved in 15 ml of 6 *N* nitric acid.

The rate of reaction of the gel with acid was studied by preparing a series of 5-g samples of gel in 125-ml glass-stoppered flasks and adding 50 ml of 0.1 *N* HCl. Samples were then placed in a water bath[1]

[1] Thermo-Shake incubator shaker, Forma Scientific, Pittsburgh, Pa.