Determination of Cyproheptadine in Plasma and Urine by GLC with a Nitrogen-Sensitive Detector

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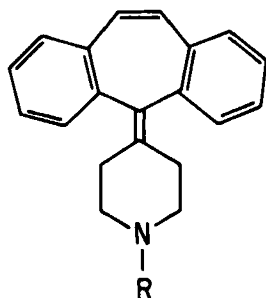
Abstract □ A method for the determination of cyproheptadine in plasma and urine was developed using the *N*-ethyl homologue as an internal standard. After extraction of the drug from an alkalinized sample into petroleum ether–isoamyl alcohol, back-extraction into 0.1 *N* HCl, washing the aqueous phase with fresh solvent, re-extraction into petroleum ether after alkalinization, the solvent was evaporated. The reconstituted residue was analyzed by GLC using a SP-2250 column and nitrogen-sensitive detector. Concentrations as low as 3 ng/ml could be determined. Plots of peak area of cyproheptadine–peak area of internal standard *versus* cyproheptadine concentration were linear over the range studied with correlation coefficients of 0.9945 and 0.9924 for plasma and urine, respectively. The method was used to determine the peak time (0.5 hr), peak concentration (33 ng/ml average), and apparent half-life (3 hr) in two dogs after oral administration of 1 mg of cyproheptadine/kg.

Keyphrases □ Cyproheptadine—GC determination in plasma and urine □ GC—cyproheptadine, determination in plasma and urine

Cyproheptadine[1], 4-(5*H*-dibenzo[*a*,*d*]cyclohepten-5-ylidene)-1-methylpiperidine (I, R = CH_3), is a potent antihistaminic and antiserotonergic agent (1) and inhibitor of platelet aggregation (2). Its disposition and metabolism in humans and animals have been described (3–9). Cyproheptadine has been quantitated in rat tissues by GC with a reported sensitivity of ~1 μg/g (3). The present report describes an analytical method suitable for the determination of cyproheptadine in low nanogram concentrations in plasma and urine.

EXPERIMENTAL

Reagents—Petroleum ether (35–60°) and all other chemicals were of analytical reagent grade and were used without further purification. Analytically pure cyproheptadine·HCl and the maleate salt of its *N*-ethyl analogue were synthesized in these laboratories. Stock solutions of the drug and the *N*-ethyl analogue used as an internal standard (II, R = C_2H_5) were prepared in methanol (1 mg/ml, as the free bases). The solution of cyproheptadine was diluted with methanol to give solutions containing 1, 0.5, 0.25, and 0.125 ng of drug/μl. The internal standard solution was diluted with methanol to give a solution containing 2 ng of the compound/μl.



I: R = CH_3
II: R = C_2H_5

A solution of isoamyl alcohol in petroleum ether (99:1, v/v) was prepared.

Instrument—Analyses were performed on a GC[2] equipped with a nitrogen-sensitive detector. The column, 0.91 m × 2 mm (i.d.) was packed with 3% SP-2250 on Supelcoport (80–100 mesh)[3]. The column was conditioned by heating overnight at 260° under helium flow (30 ml/min). Chromatographic conditions were as follows: column oven, detector, and injection port temperatures of 230, 300, and 275°, respectively; helium, hydrogen, and air flows of 30, 3.6, and 50 ml/min, respectively.

Procedure for Plasma—To 1 ml of plasma in a 13-ml glass-stoppered centrifuge tube were added 3.12, 6.25, 12.5, 25, or 50 ng of cyproheptadine and 50 ng of internal standard in a total volume of 50 μl of methanol. After addition of 1 ml of 0.1 *N* NaOH and 8 ml of petroleum ether–isoamyl alcohol (99:1), the tube was shaken for 10 min and centrifuged. Most of the organic phase was transferred to a second tube containing 1 ml of 0.1 *N* HCl. The tube was shaken for 5 min and centrifuged. The organic phase was discarded and 8 ml of petroleum ether–isoamyl alcohol added. After shaking again for 5 min and centrifuging, the organic phase was aspirated. After addition of 0.2 ml of 1 *N* NaOH and 8 ml of petroleum ether–isoamyl alcohol, the tube was shaken for 10 min and centrifuged. The solvent was transferred to a 13-ml centrifuge tube and evaporated in a stream of nitrogen at 40°. The small volume of residual isoamyl alcohol was diluted with 50 μl of heptane and 3 μl was injected into the column. The peak areas of cyproheptadine and the internal standard were measured by the chromatograph terminal and the ratio of the areas plotted *versus* the concentration of cyproheptadine present. Cyproheptadine concentrations in the unknowns were obtained by reference to this standard curve.

Procedures for Urine—The procedure was the same as for plasma except that the standard samples contained 12.5, 25, 50, and 100 ng of cyproheptadine.

RESULTS AND DISCUSSION

As shown in Fig. 1, cyproheptadine and its ethyl homologue used as an internal standard were well separated under the conditions used, having retention times of 4.2 and 5.3 min, respectively. Blank plasma samples assayed by the same procedure showed no significant interfering peaks.

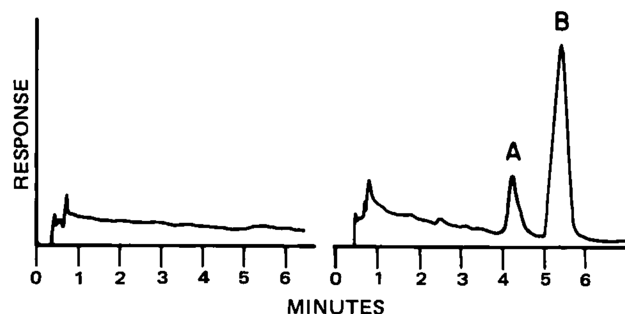


Figure 1—*Gas chromatograms of control dog plasma (left panel) and of plasma containing 12.5 ng of cyproheptadine (A) and 50 ng of internal standard (B) per milliliter (right panel).*

[1] Periactin is the registered trademark of Merck & Co., Inc. for its brand of cyproheptadine.

[2] Hewlett-Packard, Model 5840A.
[3] Supelco, Inc., Bellefonte, Pa.

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Table I—Precision and Accuracy of the GLC Assay for Cyproheptadine in Plasma and Urine

Cyproheptadine Added, ng/ml	Plasma N	Plasma Mean + SD, ng/ml	Plasma RSD, %	Plasma RE^a, %	Urine N	Urine Mean + SD, ng/ml	Urine RSD, %	Urine RE^a, %
3.12	12	3.8 ± 0.5	13.1	+21.8				
6.25	12	6.6 ± 0.7	10.6	+5.6				
12.5	12	13.2 ± 1.1	8.3	+5.6	8	11.2 ± 0.7	6.2	−10.4
25	18	24.0 ± 3.2	13.3	−4.0	8	25.4 ± 4.2	16.5	+ 1.6
50	6	48.8 ± 5.5	11.3	−2.4	8	46.9 ± 1.7	3.6	− 9.4
100					6	103.0 ± 7.3	7.1	+ 3.0

[a] Relative error.

Table II—Plasma Concentrations of Cyproheptadine in Dogs after Administration of a Single Oral Dose (1 mg/kg)

Time, hr	Plasma Concentration Dog 1, ng/ml	Plasma Concentration Dog 2, ng/ml
0.5	32	34
1	22	32
2	20	24
4	14	14
6	8	8
12	2	3

Most of the known metabolites of cyproheptadine (*i.e.*, the 10-hydroxy, 10,11-epoxy, 10-ketodesmethyl-, and 10,11-dihydroxy analogues) did not interfere since their retention times were all longer than that of cyproheptadine (7.2, 7.5, 9.3, and 10.8 min, respectively). The *N*-oxide gave a peak at the same retention time as cyproheptadine, presumably from thermal loss of oxygen during GLC. However, it was found that the *N*-oxide was not extracted from plasma or urine under these conditions and, thus, would not interfere in the analysis. Similarly, *N*-desmethylcyproheptadine had the same retention time as the internal standard, but was very poorly extracted (<10% recovery) from plasma under the conditions used. In addition, plasma and urine from dogs dosed with cyproheptadine (see below) were analyzed without addition of the internal standard. No peak was seen at the retention time of desmethylcyproheptadine, indicating that this metabolite would not interfere in the determination of cyproheptadine. However, it would be advisable before using the method in multiple-dose studies to determine if the desmethyl metabolite is present in concentrations sufficient to cause interference. If this is indicated, use of another internal standard would be required.

The precision and accuracy of the method were demonstrated by replicate analyses of plasma and urine samples containing known concentrations of cyproheptadine (Table I). The overall relative standard deviations for plasma ranged from 8.3 to 13.3% and the relative error range was −4.0–21.8%. For urine, the overall relative standard deviation range was 3.6–16.5% and the relative error −10.4–3.0%. The within-day correlation coefficient for the plasma standard curve was 0.9945 and the between-day value was 0.9825. The correlation coefficient for urine was 0.9924.

Plasma levels and urinary excretion of cyproheptadine were measured in two dogs at various times after administration of a single oral dose of the drug (1 mg/kg). The results are shown in Table II. Maximal concentrations were present at the earliest time sampled (0.5 hr). The apparent plasma half-life was ~ 3 hr. Urine (0–24 hr) contained 0.4–0.5% of the dose as unchanged cyproheptadine.

In summary, the present method was shown to be sufficiently sensitive, specific, and reliable for pharmacokinetic studies in the dog and possibly other species as well. Use of an internal standard of similar structure served to minimize errors in quantitation resulting from sample manipulation.

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