Use of Nitrogen-Specific Detector for GLC Determination of Caramiphen in Whole Blood

P. LEVANDOSKI ˣ **and T. FLANAGAN**

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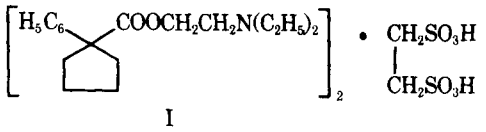
Abstract □ A sensitive GLC determination of caramiphen in whole blood was developed using a nitrogen-specific detector. The method permits the determination of levels of caramiphen as low as 2.5 ng/ml of blood and provides sufficient sensitivity and reproducibility for clinical use.

Keyphrases □ Caramiphen—analysis in whole blood by GLC with nitrogen-specific detector □ GLC—analysis, caramiphen, nitrogen-specific detector □ Antitussives—caramiphen, GLC analysis in whole blood with nitrogen-specific detector



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Caramiphen ethanedisulfonate[1] (β-diethylaminoethyl 1-phenylcyclopentane-1-carboxylate ethanedisulfonate, I) is an antitussive used as an active ingredient in cough/cold preparations.

A sensitive determination of caramiphen in blood was developed to support clinical studies. The use of a nitrogen-specific detector was proven to be specific and sensitive for other nitrogen-containing compounds in this laboratory and in reported methods for imipramine and desipramine (1), pemoline (2), and bucainide (3).

EXPERIMENTAL

Reagents—Hexane[2] was nanograde, and methanol[3] and sodium hydroxide[4] were reagent grade. Diphenylpyraline hydrochloride[5] was used as the internal standard.

All glassware was washed with chromic acid, silanized with 5% dichlorodimethylsilane[6] in toluene, and rinsed with toluene and then methanol.

Instrumentation—The gas chromatograph[7] was equipped with a nitrogen–phosphorus flame-ionization detector and a 1-mv recorder. The chromatograph was fitted with a 183-cm × 4-mm i.d. coiled glass column packed with 3% OV-17 on 80–100-mesh Chromosorb WHP[6]. The column was conditioned overnight at 310° with a helium flow and several 25-μl injections of a silylating agent[8]. Normal operating temperatures were 270, 300, and 300° for the oven, injection port, and manifold, respectively. The recorder speed was 20 mm/min. Helium was used as the carrier gas at a flow rate of 60 ml/min. The air gauge was set at 3.5 kg/cm². The detector was run in the nitrogen mode at a setting of 710.

Preparation of Standards—Standard stock solutions of 10 mg/100 ml (as the free base) of caramiphen and the internal standard were pre-

Table I—Reproducibility of Caramiphen Standards

Blood Concentration, ng/ml	*n*	Mean Peak Height Ratio ± *SE*	*SE*, %
2.5	2	0.05 ± 0.004	6.7
5	4	0.12 ± 0.012	10.0
10	5	0.28 ± 0.010	3.6
15	4	0.43 ± 0.021	4.9
20	5	0.59 ± 0.011	1.9
30	5	0.93 ± 0.008	0.9
40	4	1.21 ± 0.051	4.2

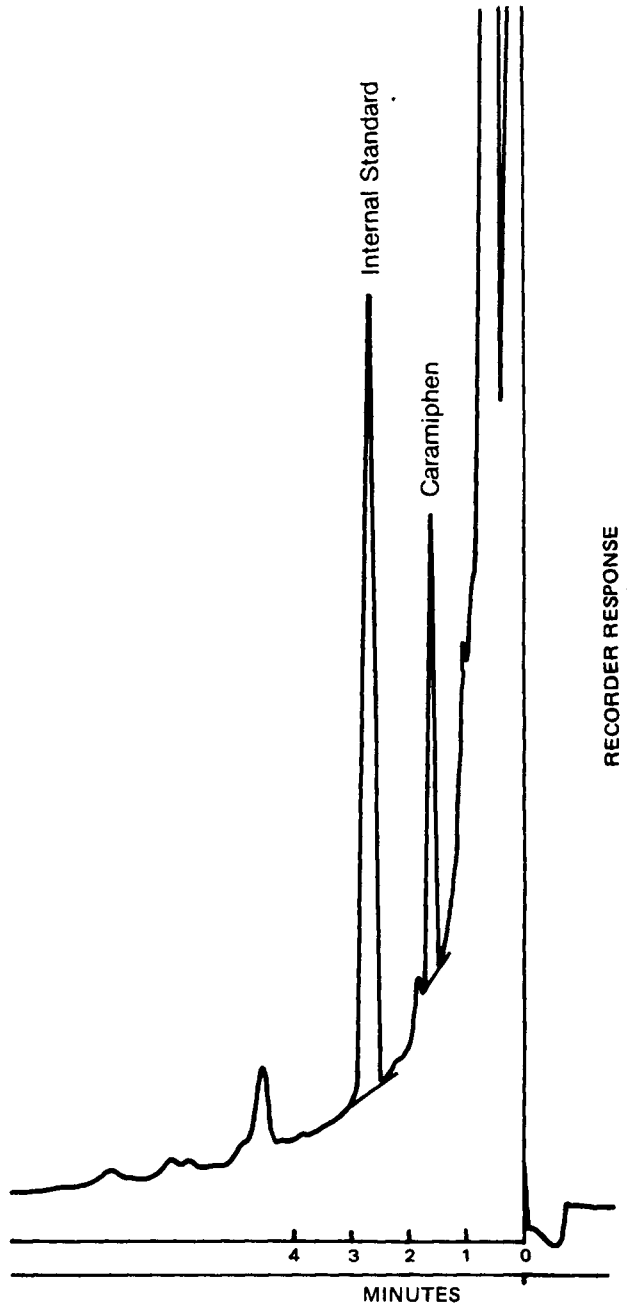

Figure 1—*Chromatogram of a control blood sample that was spiked with the caramiphen reference standard and the internal standard. The peak at 1.6 min represents 20 ng of caramiphen/ml whereas the internal standard (total of 250 ng) has a retention time of 2.7 min.*

[1] Synthesized at SmithKline Corp.
[2] Mallinckrodt.
[3] J. T. Baker Chemical Co.
[4] Fisher Scientific Co.
[5] Napco Chemical Co.
[6] Supelco Inc.
[7] Perkin-Elmer model 3920.
[8] Rejuv-8, Supelco Inc.

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Table II—Blood Levels of Caramiphen in 11 Subjects Receiving 20 mg of Caramiphen Edisylate at 0, 4, and 8 hr Daily for 4 Consecutive Days

Subject	Concentration of Caramiphen, ng/ml											
	0 hr	1 hr	2 hr	4 hr	5 hr	6 hr	8 hr	9 hr	10 hr	13 hr	18 hr	24 hr
1	7.0	43.5	43.5	20.0	74.5	65.0	20.3	21.3	46.0	31.0	13.5	7.5
2	2.5	4.0	11.5	4.5	9.3	17.5	5.0	9.5	12.8	5.8	2.5	3.3
3	3.3	27.8	18.0	9.5	16.0	45.5	17.0	21.0	40.0	14.0	5.3	2.5
4	<1	3.4	31.4	14.6	24.8	21.5	7.9	7.0	21.5	6.3	2.8	1.2
5	9.6	46.6	32.7	15.5	64.5	48.5	21.0	25.0	53.0	22.5	11.6	7.0
6	2.7	16.4	10.6	4.0	5.7	27.8	6.8	4.0	4.0	6.3	7.0	2.0
7	3.5	25.0	23.0	9.0	27.0	34.0	14.0	10.0	29.0	10.0	4.0	3.0
8	5.3	6.0	57.3	29.0	28.5	78.0	30.5	41.5	59.5	36.0	15.5	6.0
9	3.0	15.5	26.0	11.0	28.0	19.0	9.8	10.0	24.5	13.5	6.8	4.0
10	3.0	21.8	27.0	16.5	47.0	34.5	13.0	63.8	34.8	11.5	7.5	4.0
11	2.5	11.5	11.0	3.5	3.3	23.0	10.5	25.0	18.0	5.0	2.8	2.0
Average	3.9	20.1	26.5	12.5	29.9	37.7	14.2	21.6	31.2	14.7	7.2	3.9

pared in methanol. A working internal standard solution (0.5 μg/ml) was prepared by diluting 1 ml of the stock solution to 200 ml with distilled water. A working standard caramiphen solution (100 ng/0.1 ml) was prepared by diluting 1 ml of the stock solution to 100 ml with distilled water.

Extraction Procedure—Aliquots of 0, 12.5, 25, 50, 75, 100, 150, 200, 300, and 400 μl of the working standard solution of I were added to 5 ml of blank whole blood in 12-ml silanized test tubes. A 0.5-ml aliquot of the working internal standard solution was added to each tube. These samples were used to generate a standard curve. A 0.5-ml aliquot of the internal standard solution was added to all unknown samples (5-ml aliquots).

The pH was adjusted to 9.0 with 5% NaOH. A 1-ml aliquot of hexane was added, and the tubes were stoppered and shaken slowly (65 shakes/min) on an automatic shaker[9] for 30 min and centrifuged[10] for 10 min. The hexane layer was transferred to a second 12-ml silanized test tube, and the blood was extracted with another 1-ml aliquot of hexane with shaking for 30 min and centrifugation for 10 min. The second hexane extract was added to the first extract, and the combined solution was evaporated to dryness under a nitrogen stream. The residue was dissolved in 25 μl of methanol. Aliquots (2 μl) were injected into the gas chromatograph.

Calculations—A calibration curve was constructed by plotting the peak height ratio of caramiphen to the internal standard against the blood concentration of caramiphen on linear coordinate paper. Unknown values were read from the calibration curve.

RESULTS AND DISCUSSION

Figure 1 is a typical chromatogram of a spiked blood sample used for the standard curve. The peak at 1.6 min represents 20 ng of caramiphen/ml, and the peak at 2.7 min represents the internal standard (total of 250 ng added).

Spiked standards over the range of 2.5–40 ng/ml were analyzed. Table I shows the standard deviation and standard error obtained.

The assay sensitivity was <2.5 ng/ml of blood. Calibration curves were linear over the range of 2.5–80 ng of caramiphen/ml of blood.

Caramiphen is stable in frozen whole blood specimens for at least 1 week. Its stability was not studied beyond this period.

After overnight fasting, 11 subjects received 20 mg of caramiphen edisylate (plain drug in capsule) at 0, 4, and 8 hr daily for 4 consecutive days. On the 4th day, blood was drawn in heparinized syringes at 0, 1, 2, 4, 5, 6, 8, 9, 10, 13, 18, and 24 hr. The levels of caramiphen found are shown in Table II.

All 11 blood samples were screened before drug administration, and no interference peaks appeared in the area of caramiphen or its internal standard. These control bloods were spiked with the caramiphen standards and the internal standard. Based on these studies, the method was judged as specific for the determination of caramiphen in blood from controlled clinical studies when the subjects were free from other medication.

The sensitivity, reproducibility, and specificity make this method desirable for bioavailability and pharmacokinetic studies.

[9] Arthur H. Thomas Co.
[10] International, Arthur H. Thomas Co.

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