Capillary GLC Assay for Carbinoxamine and Hydrocodone in Human Serum Using Nitrogen-Sensitive Detection

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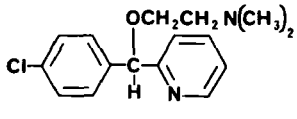
Abstract □ Capillary gas chromatography using an open tubular fused silica column and NP-FID was applied to the simultaneous analysis of the antihistamine, carbinoxamine, and the antitussive, hydrocodone, in human serum. Carbinoxamine and hydrocodone were extracted into methylene chloride–2-propanol (9:1) under alkaline conditions along with their respective internal standards, brompheniramine and *N*-ethylhydrocodone. The basic drugs were back-extracted into 0.1 *N* sulfuric acid and reextracted into benzene after making the aqueous phase alkaline with potassium hydroxide. The benzene extracts were evaporated to dryness and the residues were reconstituted with 40 μl of *n*-nonyl alcohol–methanol (19:1). Samples (1–2 μl) were injected onto the capillary column in the splitless mode (solvent effect) at 185° and the temperature programmed to 250°. Calibration curves using spiked serum standards were linear to at least 20 ng/ml for both drugs. Coefficients of variation averaged ±6.1% for carbinoxamine and ±5.0% for hydrocodone in the 2–15 ng/ml range. Sensitivity was estimated to be ~0.2 ng/ml for a 2-ml serum sample. Serum levels of carbinoxamine and hydrocodone were determined in a human volunteer administered these drugs.

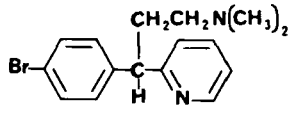
Keyphrases □ GLC assay—carbinoxamine and hydrocodone in human serum, nitrogen sensitive detection □ Carbinoxamine—GLC assay, nitrogen sensitive detection □ Hydrocodone—GLC assay, nitrogen sensitive detection □ Nitrogen sensitive detection—GLC assay for carbinoxamine and hydrocodone in human serum

Carbinoxamine, 2-[(4-chlorophenyl)-2-pyridinylmethoxy]-*N*,*N*-dimethylethanamine, is an antihistamine structurally related to chlorpheniramine. Quantitative methods for determining carbinoxamine in biological fluids have not been published. However, several methods have been published for chlorpheniramine. These methods include GLC–MS (1, 2), GLC–electron-capture detection (3), GLC–nitrogen-sensitive detection (4, 5) and high-performance liquid chromatography (HPLC) (6, 7).

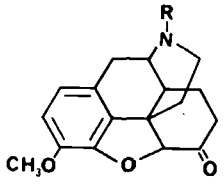
Hydrocodone, 4,5α-epoxy-3-methoxy-17-methylmorphinan-6-one, is a widely used antitussive agent. A method to determine hydrocodone in serum using GLC–electron-capture detection has been described by Barnhart and Caldwell (8) using the pentafluorophenylhydrazone derivative. The purpose of this investigation was to develop a specific and sensitive capillary GLC method for the simultaneous determination of carbinoxamine and hydrocodone in human serum.



CARBINOXAMINE



BROMPHENIRAMINE (INTERNAL STANDARD)



R = -CH_3 HYDROCODONE
R = -C_2H_5 N-ETHYLHYDROCODONE (INT.STD.)

EXPERIMENTAL

Chemicals—Carbinoxamine maleate, hydrocodone bitartrate, and brompheniramine maleate were USP reference standards. Reagent grade potassium hydroxide, sulfuric acid, hydrochloric acid, and 2-propanol were used without further purification[1]. Methylene chloride, benzene, and methanol were glass distilled[2]. *n*-Nonyl alcohol[3] was distilled in glass and the fraction boiling between 214°–215° was used.

Instrumentation—The GLC analyses were performed on a gas chromatograph[4] equipped with a capillary injection system[5] and a nitrogen-sensitive detector. Separations were performed on a 10–12 m × 0.25-mm i.d. fused silica open tubular column wall-coated with SE-30[6]. The film thickness was 0.25 μm. The flow rate of the helium carrier gas was adjusted to give partition ratios (k) of ~4.6 and 8 for carbinoxamine and hydrocodone, respectively, under the chromatographic conditions described below. Linear flow rates were in the 30–40-cm/sec range. The initial oven temperature was 185°. After 1.1 min at 185° it was programmed to increase at 25°/min for 0.9 min and then at 10°/min until it reached 250° where it was held for 1 min. The temperature of the nitrogen-specific detector was 300°, with hydrogen and air flow rates of 3.0 and 50 ml/min, respectively. The injection port temperature was 260°. A 8 cm × 2-mm i.d. fused silica splitless liner was used with a purge activation time of 0.9 min. The computing integrator had a slope sensitivity of 0.1 and an attenuation of 2^3 or 2^2.

Preparation of the Hydrocodone Internal Standard—The internal standard for hydrocodone, 4,5-epoxy-3-methoxy-17-ethylmorphinan-6-one, herein referred to as *N*-ethylhydrocodone, was synthesized from dihydronorcodeinone by the method of Clark and Woodbridge (9). Dihydronorcodeinone was synthesized from hydrocodone using the cyanogen bromide method of Clark *et al.* (10).

Preparation of Serum Standards—About 35 mg of carbinoxamine maleate and 37.5 mg of hydrocodone bitartrate reference standards were accurately weighed into a 50-ml volumetric flask and dissolved in water. A secondary aqueous standard was made in water to a final concentration of ~10 μg/ml as the free base. Working serum standards at 15, 10, 5, and 2 ng/ml were prepared by adding 75, 50, 25, and 10 μl of the secondary standard to a 50-ml volumetric flask and adjusting to volume with drug-free serum. The serum should be checked by the analytical procedure for interferences, since antihistamines are available in common nonprescription cold preparations. Internal standards, brompheniramine and *N*-ethylhydrocodone, were prepared in the same solution of 0.01 *N* HCl at 40 ng/ml.

Extraction Procedure—To a screw-cap test tube was added 2 ml of serum sample or standard, 1 ml of internal standard solution, 1 ml of 2 *N* KOH, and 6 ml of methylene chloride–2-propanol (9:1). The test tube

[1] Mallinckrodt reagent grade.
[2] Burdick and Jackson.
[3] Eastman Chemical Co.
[4] Hewlett-Packard Model 5840A.
[5] Hewlett-Packard Model 18835B.
[6] J & W Scientific, Inc.

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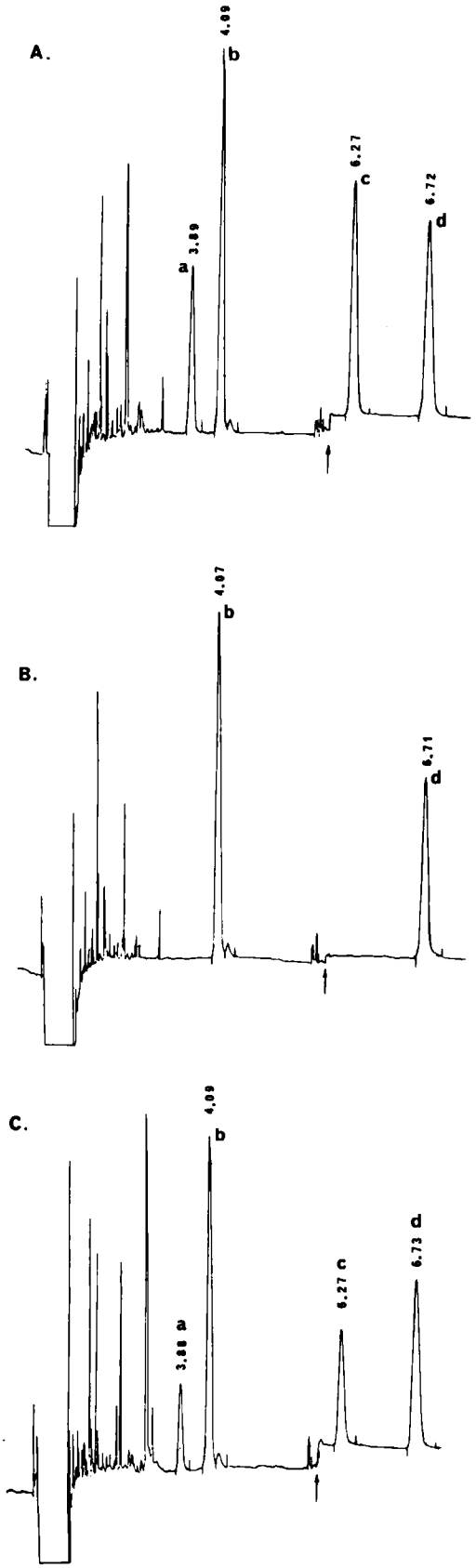

Figure 1—*Chromatograms of serum extracts. Key: (A) Serum standard containing 10 ng/ml of carbinoxamine and hydrocodone; (B) Serum sample of human volunteer prior to drug administration; (C) Serum sample of human volunteer 4.5 hr postdose with 5 mg of hydrocodone bitartrate and 4 mg of carbinoxamine maleate; (a) carbinoxamine; (b) brompheniramine; (c) hydrocodone; (d) N-ethylhydrocodone. Arrow denotes attenuation change from 2^3 to 2^2.*

Table I—Analytical Precision of the Determination of Carbinoxamine and Hydrocodone in Spiked Human Serum

Concentration, ng/ml	Peak Area Ratio[a] (n = 4)	Calculated Concentration, ng/ml[a]	*RSD*
Carbinoxamine			
0	0	0.5	—
2	0.122 ± 0.012	1.97 ± 0.19	9.6
5	0.302 ± 0.019	4.81 ± 0.29	6.1
10	0.651 ± 0.030	10.3 ± 0.48	4.7
15	0.940 ± 0.038	14.9 ± 0.61	4.1
Hydrocodone			
0	0	−0.006	—
2	0.221 ± 0.16	2.03 ± 0.15	7.4
5	0.530 ± 0.032	4.87 ± 0.29	6.0
10	1.11 ± 0.038	10.2 ± 0.36	3.5
15	1.62 ± 0.050	14.9 ± 0.46	3.1

[a] Mean ± *SD*.

was capped and shaken at a moderate rate (150 cpm) on a reciprocal shaker[7] for 10 min. After centrifugation[8] at 2500 rpm for 5 min at 2–5°, the upper aqueous phase was discarded by aspiration. The organic phase was decanted into a clean test tube containing 2 ml of 0.1 *N* sulfuric acid and capped. After shaking and centrifuging the upper aqueous layer was transferred to a test tube containing 0.3 ml 2 *N* KOH and 2 ml of benzene. The sample was vigorously mixed[9] for 10 sec and centrifuged. Avoiding any aqueous phase, the benzene layer was transferred with a pipet freshly rinsed with absolute ethanol to a conical screw-cap test tube. The sample was evaporated to dryness at 35°–40° with a gentle stream of dry, filtered air. The residue was constituted with 40 μl of 5% methanol in *n*-nonyl alcohol and the entire test tube wall was rinsed with the solvent and centrifuged. A 2–3 μl aliquot was injected into the GLC using the splitless injection technique.

Injection Technique—Since injections were made in the splitless mode, using the solvent effect (11), the injection process was slow. The sample was loaded into a 10-μl syringe followed by air to the 1-μl mark. The purge flow was eliminated and the septum was pierced by the syringe needle. The needle was heated for 5–10 sec in the injection port and the sample injected at a rate of ~1 μl/sec. The syringe needle was left in the injection port for 5 sec after sample introduction.

Calculations—Calibration curves of peak area ratios (carbinoxamine–brompheniramine and hydrocodone–*N*-ethylhydrocodone) *versus* serum carbinoxamine or hydrocodone concentrations were linear to at least 20 ng/ml. Unknowns were calculated from a least-squares linear regression fit to known carbinoxamine and hydrocodone serum standards.

RESULTS AND DISCUSSION

Capillary GLC is inherently more sensitive than conventional GLC because of the higher amount of detectable substance passing through the detector per unit of time. Sensitivity is of particular importance in the analysis of drugs such as carbinoxamine and hydrocodone where peak serum concentrations are typically in the 5–15-ng/ml range.

Chromatographic peaks of carbinoxamine and hydrocodone were symmetrical and well resolved from serum coextractives, as shown in Fig. 1. It was necessary to use two internal standards because of the different extraction and chromatographic properties of carbinoxamine and hydrocodone. Brompheniramine was chosen as the internal standard for carbinoxamine because it eluted in a clean chromatographic region and was completely resolved from carbinoxamine. Chlorpheniramine, which elutes just prior to carbinoxamine, was not used because of its more common presence in pharmaceutical antihistamine preparations. This method could be used to determine serum chlorpheniramine levels. Either carbinoxamine or brompheniramine would suffice as an internal standard for a chlorpheniramine assay.

The precision of both assays is shown in Table I. In the concentration range of 2–15 ng/ml the coefficients of variation ranged from 4.1 to 9.6% for carbinoxamine and from 3.1 to 7.4% for hydrocodone. Extraction efficiencies averaged 71.2 ± 2.8% and 78.4 ± 2.5% for carbinoxamine and hydrocodone, respectively, at 10 ng/ml.

[7] Eberbach Corp.
[8] Sorvall RC-3B, DuPont, Inc.
[9] Maxi Mix Model M-16715, Thermolyne, Sybron Corp.

Table II—Analysis of Spiked Serum Samples of Carbinoxamine and Hydrocodone at Low Concentration

Concentration, ng/ml	Peak Area Ratios[a]	
	Carbinoxamine/IS	Hydrocodone/IS
0.2	0.0065 ± .0018 (28.2%)[b]	0.0088 ± .002 (25.9%)
0.5	0.0206 ± .0042 (20.3%)	0.0423 ± .0061 (14.4%)
1.0	0.0736 ± .0075 (10.2%)	0.091 ± .0079 (8.7%)

[a] Mean ± *SD*; $n = 4$. [b] Coefficient of variation (%).

Calibration curves of peak area ratios *versus* concentration in serum were linear up to at least 20 ng/ml. Assay sensitivity or the minimum quantifiable concentration was evaluated by determining the assay precision in the 0.2–1.0-ng/ml range. By defining the sensitivity as that concentration of drug that has a ±25% *CV*, the sensitivity for both carbinoxamine and hydrocodone is ~0.2 ng/ml using a 2-ml sample volume (Table II).

To assess the validity of this analytical procedure, serum samples from a human volunteer were assayed. The serum carbinoxamine and hydrocodone concentrations from a human volunteer orally administered, under fasted conditions, a solution containing 4 mg of carbinoxamine maleate and 5 mg of hydrocodone bitartrate are shown in Table III. Typical chromatograms from a human volunteer are also shown in Fig. 1. This assay provides the precision and sensitivity to conduct pharmacokinetic and bioavailability studies in humans receiving a single oral dose of carbinoxamine maleate and hydrocodone bitartrate.

Table III—Serum Carbinoxamine and Hydrocodone Concentrations after Oral Administration of 4 mg of Carbinoxamine Maleate and 5 mg of Hydrocodone Bitartrate to a Human Volunteer

Time Postdose, hr	Serum Concentration, ng/ml	
	Carbinoxamine	Hydrocodone
0	0	0
0.5	1.1	4.6
1	3.8	9.1
1.5	4.6	10.7
2	8.0	10.4
3	7.5	7.5
4.5	6.3	4.3
6	5.5	4.2
9	3.2	1.7
12	1.7	0.6
24	1.1	0
36	0.7	0

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Hypolipidemic Activity of Phthalimide Derivatives IV: Further Chemical Modification and Investigation of the Hypolipidemic Activity of *N*-Substituted Imides

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Abstract □ A further investigation of *N*-substituted derivatives of phthalimide for hypolipidemic activity has revealed that the chain length, as well as the type of substitution on the *N*-alkyl chain of phthalimide is critical for biological activity. In these studies the hypolipidemic activity was not improved by extending the chain length beyond five carbon atoms in the alkyl and alkanoic acid series. Imido nitrogen substituents, other than alkanoic acids, methyl ketones, and alkyl groups, caused a reduction in hypolipidemic activity, *e.g.*, hydroxy, amino, hydroxymethyl, or carbethoxy. Reduction of the keto group in the side chain to an alcohol, as well as forming derivatives of the keto group, did not improve the hypolipidemic activity with the exception of 1-*N*-phthalimidobutan-3-one semicarbazone. This compound demonstrated improved hypocholesterolemic activity over phthalimide and 1-*N*-phthalimidobutan-3-one. Substitution of the 3-position of the aromatic moiety of phthalimide with an amino or nitro group, as well as substituting a pyridine or cyclohexyl ring for the phenyl ring, led to the loss of hypolipidemic activity.

Keyphrases □ Phthalimide—*N*-substituted derivatives, synthesis, hypolipidemic activity, mice, structure–activity relationships □ Hypolipidemic agents—potential, *N*-substituted derivatives of phthalimide, structure–activity relationships, mice □ Structure–activity relationships—*N*-substituted phthalimide derivatives, hypolipidemic activity, mice

Previously, it was shown that phthalimide (I) is a potent hypolipidemic agent in rodents. Serum cholesterol levels were reduced 43% in mice after administration for 16 days at 20 mg/kg/day. Serum triglyceride levels were also reduced 56% after 14 days of administration in mice at the same dose. The phthalimide derivatives were more potent

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