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GAS CHROMATOGRAPHIC DETERMINATION OF HYDROCODONE IN SERUM

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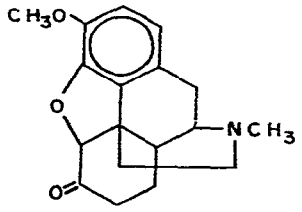
SUMMARY

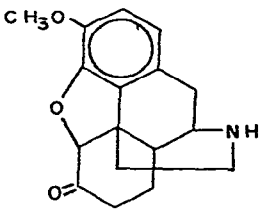
A procedure for the determination of hydrocodone (dihydrocodeinone) in serum has been developed. Hydrocodone and N-isobutyldihydronorcodeinone, the internal standard, are extracted from serum by chloroform–isopropyl alcohol (9:1, v/v). The extracts are purified by back-extraction into 0.1 *N* sulfuric acid and a final basic extraction into benzene. The pentafluorophenylhydrazone derivatives are formed and determined using electron capture gas chromatography. As little as 1 ng/ml of hydrocodone in serum can be determined. A closely related compound and potential metabolite, dihydronorcodeinone, does not interfere. Serum hydrocodone levels were determined in dogs after oral and intravenous doses of 0.5 mg/kg, and in humans after a 10-mg oral dose of the bitartrate. A mean peak serum drug concentration of 23.6 ng/ml and a terminal half-life of 3.8 h resulted from the human study. The terminal half-life in serum was 1.8 h after the intravenous dose in dogs.

INTRODUCTION

Hydrocodone (dihydrocodeinone) is a widely used antitussive agent[1]. Little is known about the pharmacokinetic or metabolic characteristics of this drug in spite of its widespread usage. Although the determination of hydrocodone (I) in biological samples was mentioned in the literature, it was in the context of a screening procedure for forensic purposes and no data were reported[2,3]. These procedures lacked the sensitivity to determine the drug in serum after therapeutic doses. A new gas chromatographic procedure for the determination of the drug in serum with a lower limit of 1



I



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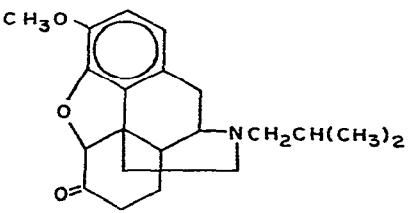

III

ng/ml was devised utilizing electron capture detection of the pentafluorophenylhydrazone derivative. A similar approach has been used for the determination of plasma estrone[4].

EXPERIMENTAL

Instrumentation

A Hewlett-Packard Model 7610A gas chromatograph (Hewlett-Packard, Avondale, Pa., U.S.A.) with a ^{63}Ni electron capture detector was used with a 3 ft. × 4 mm I.D. silanized glass column filled with commercially available packing, 3% OV-7 on Supelcoport 100–120 mesh (Supelco, Bellefonte, Pa., U.S.A.). The column oven temperature was 265°, and the injection port and detector temperatures were 280° and 300°, respectively. The carrier gas (5% methane in argon) flow-rate was 40 ml/min. The pulse interval for the electron capture detector was 150 μsec. Under these conditions, the pentafluorophenylhydrazones of compounds I, II, and III had retention times of 9.4, 10.6, and 12.9 min, respectively.

Reagents

All organic solvents listed in the procedure were nanograde quality (Mallinckrodt, St. Louis, Mo., U.S.A.). Pentafluorophenylhydrazine (PFPH) was obtained from Regis (Morton Grove, Ill., U.S.A.), hydrocodone bitartrate and methadone hydrochloride from Mallinckrodt, and oxycodone hydrochloride and hydromorphone hydrochloride from Merck (Rahway, N.J., U.S.A.).

N-Isobutyldihydronorcodeinone (III) and dihydronorcodeinone (II) were synthesized from hydrocodone bitartrate as the starting material using published procedures[5,6]. Compound III was freed from small amounts of contaminating compound II by passing a methanolic solution of compound III free base through a 1 × 8 cm silicic acid column (Bio-Sil HA; Bio-Rad Lab., Richmond, Calif., U.S.A.) and eluting with methanol. Compound III eluted in the first 20 ml and compound II was retained on the column. A working solution was prepared by diluting 0.10 ml of the methanolic solution containing approximately 1 mg/ml to 100 ml with 0.01 *N* sulfuric acid. All other reagents were analytical-reagent grade.

Procedure

A sample containing 1–2 ml of serum, internal standard, 0.5 ml of 2 *N* potassium hydroxide, and 2 ml of distilled water was extracted with 4.5 ml of chloroform–isopropyl alcohol (9:1, v/v) for 5 min. The aqueous phase was discarded with the aid of centrifugation and the organic phase was decanted into another tube. Basic substances in the organic phase were extracted with 2 ml of 0.1 *N* sulfuric acid by mixing for 1 min on a rotary mixer. After centrifugation, the aqueous layer was transferred to another tube, 0.2 ml of 2 *N* potassium hydroxide was added, and the sample was mixed vigorously with 2 ml of benzene for 1.5 min on a rotary mixer. The sample was centrifuged and the benzene layer was transferred to a small test tube. Solvent was evaporated with nitrogen in a warm (50°) sand bath. Derivatization was accomplished by adding 0.1 ml of 1% (v/v) acetic acid in methanol and 0.1 ml of PFPH in methanol (2 mg/ml). After 2 h at room temperature, 2 ml of water and 0.1 ml of 2 *N* potassium hydroxide were added, and the sample was mixed vigorously with 2 ml of benzene for

1 min on a rotary mixer. The benzene was transferred after centrifugation to a small vial and evaporated to dryness. The residue was dissolved in 50–100 μl of benzene–methanol (9:1, v/v). At least three standards in serum were carried through the procedure and all standards and samples were done in duplicate. Peak height ratios (I/III) were calculated and plotted *versus* the concentration of I for the standard curve. A typical standard curve is shown in Fig. 1.

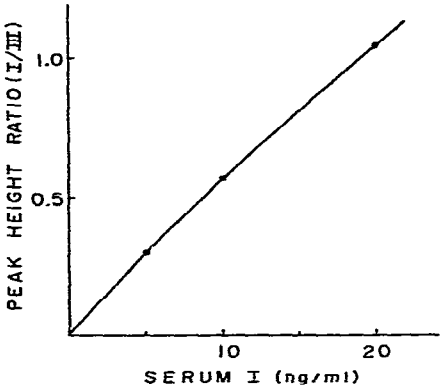

Fig. 1. Standard curve for the quantitative analysis of hydrocodone in serum.

Dog studies

Two male (No. 4258 and 986) and two female (No. 898 and 863) mongrel dogs weighing 21.3, 26.5, 23.7, and 20.8 kg, respectively, received a single intravenous (i.v.) dose of 0.5 mg/kg of I bitartrate (USP) in 0.9% saline via the cephalic vein. The animals were fasted for 12 h prior to and during the experiment, but had free access to water. Blood samples (15 ml) were taken at selected intervals from 0 through 6 h after dosing. Serum was collected and stored at −15°. One week later the experiment was repeated in the same animals using oral intubation of the drug solution.

Human studies

Five male human subjects from 21 to 26 years old, weighing 70, 61, 59, 77, and 77 kg were given a single 10-mg oral dose of I bitartrate. The formulation, a commercial tablet, also contained 60 mg of pseudoephedrine·HCl. The subjects were fasted overnight before dosing, but were given a light snack 2 h after dosing and lunch 5 h after dosing. The diet was not supervised after 8 h. Blood samples (20 ml) were taken at selected intervals from 0–12¼ h after dosing. Serum was collected and stored at −15°.

RESULTS AND DISCUSSION

Test analyses

A series of analyses of control serum containing known concentrations of hydrocodone was performed as a test of the reproducibility of the method (Table I).

TABLE I

REPLICATE ANALYSES OF CONTROL SERUM CONTAINING KNOWN AMOUNTS OF HYDROCODONE

Actual concentration of hydrocodone in serum (ng/ml)	*Concentration of hydrocodone found in serum (ng/ml)*	*Mean (ng/ml)*	*Coefficient of variation (%)*
0	0.0, 0.0, 0.1, 0.0	0.0	0.0
1.25	1.1, 1.3, 1.2*, 1.3*	1.2	7.9
7.5	7.5, 7.6, 7.4*, 7.5*	7.5	1.1
8.5	8.5, 8.3, 7.9*, 8.0*	8.2	3.4
10.0	10.3, 10.2, 9.9, 10.5, 10.2, 9.4, 9.2, 9.7, 10.2, 9.7	9.9	4.3
16.0	14.5, 14.2, 15.6*, 15.7*	15.0	5.1
21.0	20.2, 21.7, 21.6*, 19.0*	20.6	6.2
25.0	25.1, 24.9, 25.2, 25.0, 26.0, 23.5, 25.2, 25.0, 24.9, 25.4	25.0	2.5

* 2.0 ml serum sample.

There was no material difference in the results obtained using 1- or 2-ml serum samples, so these data were combined for statistical analysis. An overall accuracy of 98.5% of theoretical was obtained, with a coefficient of variation of 4.4%.

Specificity

Compounds lacking a basic functional group would not be expected to survive the extraction techniques used in this procedure. Hydromorphone, a compound with both basic and acidic functional groups, did not interfere when tested at the 1-μg/ml level, and, in fact, was not extracted at the high pH used in this method. Direct derivatization of hydromorphone produced a compound with a relative retention of 0.67 compared to the derivative of I. Methadone was also tested at the 1-μg/ml level, and did not interfere. Oxycodone was readily detected and could perhaps be used as an internal standard, since it and III formed derivatives with the same retention time. However, the oxycodone peak had considerably more tailing.

Analytical conditions

Hydrocodone is a weak base and can be extracted from aqueous solution with organic solvents at elevated pH. Chloroform and benzene were found to be good extracting solvents whereas hexane and diethyl ether were poor. Back extraction of the amines with dilute acid and subsequent extraction of the free amines after pH adjustment was found to be an adequate cleanup of serum extracts. Derivatization with PFPH required a small amount of acid and an extended time period for adequate reaction (Table II). The conditions selected were 0.5% acetic acid concentration, 1.0-mg/ml PFPH concentration, and a 2-h reaction time. The PFPH derivative of the internal standard (III) was separated chromatographically from those of both I and a potential metabolite (II). Peak height ratios (I/III) were plotted *versus* concentration of I (Fig. 1) to yield linear or nearly linear standard curves. II was not quantitated, but the peak heights were substantially less than those for equal quantities of I. Whether this was due to an actual difference in sensitivity or poor recovery was not determined. Some representative chromatograms are shown in Fig. 2.

TABLE II

CONDITIONS FOR FORMATION OF HYDROCODONE PENTAFLUOROPHENYLHYDRAZONE

(A), 50 μg hydrocodone in 0.4 ml methanol with 1 mg PFPH, flame ionization detection of hydrocodone and derivative peaks, overnight reaction time (effect of acetic acid concentration). (B), the same as (A), except reaction time and with 0.5% acetic acid (effect of reaction time). (C) 100 ng hydrocodone in 0.2 ml methanol, 0.5% acetic acid, 2 h reaction, electron capture detection of derivative (effect of PFPH concentration).

A		*B*		*C*	
Acetic acid (%)	*Hydrocodone/ derivative peak area ratio*	*Time (h)*	*Hydrocodone/ derivative peak area ratio*	*PFPH concentration (mg/ml)*	*Derivative peak height (cm/ng hydrocodone)*
0.25	0.04	0.50	0.38	0.125	6.1
0.50	0.05	1.00	0.14	0.25	10.7
1.25	0.08	2.25	0.04	0.50	14.2
2.50	0.17	4.00	0.05	1.00	20.8
3.75	0.27			1.50	21.3
5.00	0.30			2.00	20.6
				2.50	19.0

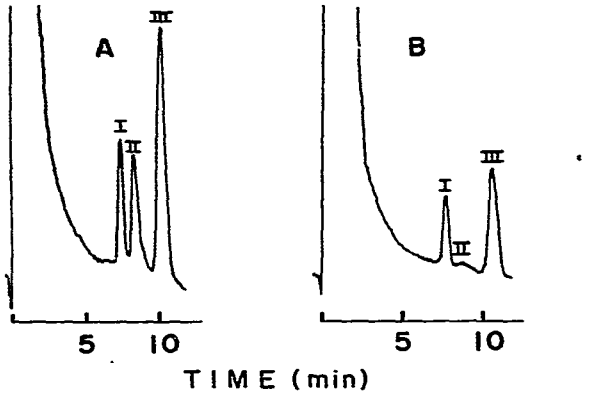

Fig. 2. Representative chromatograms from canine and human serum analyses. (A), Dog no. 898, oral administration, 4 h, 200 ng of compound III; (B), subject no. 112, oral administration, 5 h, 75 ng of compound III.

Dog study

Serum drug levels were determined after the oral and i.v. administration of I bitartrate to four dogs (Table III). In general, the data were quite consistent except during the oral absorptive phase, when considerable variability was discovered. Serum I levels fell rapidly after i.v. administration, decreasing to less than 10% of maximum values after 6 h. The terminal half-life calculated from the 2–6-h data, was 1.7 ± 0.1 (S.D.) h. The complete curve is typical of a two-compartment open model[7] (Fig. 3). Oral absorption was rapid, with the mean peak value of 66 ng/ml occurring at 0.9 h. After attainment of first-order disappearance, the half-life was 1.8 ± 0.4 h. The area under the curve through 6 h was calculated using the trapezoidal rule. Values of 171 and 143 $ng \cdot ml^{-1} \cdot h$ were obtained for the i.v. and oral routes, respectively. The 84%

TABLE III

SERUM HYDROCODONE LEVELS AFTER I.V. OR ORAL ADMINISTRATION OF HYDROCODONE BITARTRATE (0.5 mg/kg) IN DOGS

Time (h)	*Serum hydrocodone (ng/ml, as the free base)*					
	Dog No.					
	4258	*986*	*898*	*863*	*Mean*	*S.D.*
Intravenous						
0	0.3	0.2	0.6	0.3	0.4	0.2
¼	106	103	107	106	106	1.7
½	86	68	86	86	82	9.0
¾	70	61	74	68	68	5.4
1	61	55	64	50	58	6.2
1½	37	38	46	33	38	5.4
2	31	33	34	24	30	4.5
3	22	24	22	15	21	3.9
4	10	12	12	10	11	1.2
6	5.4	7.1	6.4	4.9	6.0	1.0
Oral						
0	0.0	0.0	0.0	0.0	0.0	0.0
¼	52	1.8	7.3	0.8	15	25
½	94	5.4	42	25	42	38
¾	78	10	69	68	56	31
1	68	26	62	60	54	19
1½	48	33	40	45	42	6.6
2	36	28	32	32	32	3.3
3	23	16	23	24	22	3.7
4	14	10	15	17	14	2.9
6	5.6	8.0	5.6	7.9	6.8	1.4

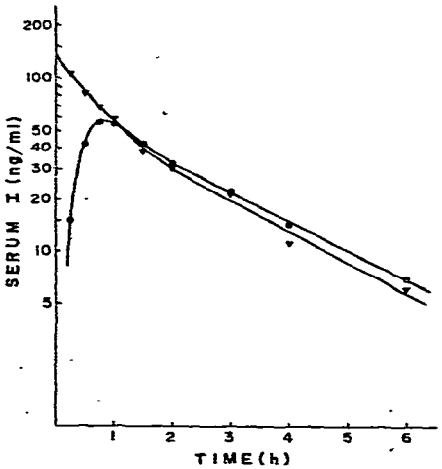

Fig. 3. Mean serum hydrocodone levels in four dogs after a single intravenous (▼) or oral (●) dose.

oral availability is indicative of good absorption and no major first pass metabolic effect.

The metabolism of hydrocodone has not been reported; however, by analogy to similar compounds, such as morphine and codeine[8], N-demethylation would be one potential route. Although the present study was not primarily a metabolic study, the N-demethylated compound (II) was separated chromatographically and could therefore be detected. A component with the same elution time as II was detected in some serum extracts (Fig. 2). This peak was more pronounced after oral administration. Quantitation was not attempted, but it should be remembered that the sensitivity and/or recovery of II was poor.

Human study

Considering that the dose in human was about 0.14 mg/kg the observed serum drug levels (Table IV) compare quite favorably with those obtained in the dog. The mean peak concentration was 23.6 ± 5.2 ng/ml while the corresponding canine value

TABLE IV

SERUM HYDROCODONE LEVELS AFTER ORAL ADMINISTRATION OF HYDROCODONE BITARTRATE (10 mg) IN HUMANS

Time (h)	*Serum hydrocodone (ng/ml, as the free base)*						
	Subject					*Mean*	*S.D.*
	103	*106*	*107*	*110*	*112*		
0	0.2	0.0	0.2	0.0	0.0	0.1	0.1
½	6.6	6.4	12	6.0	7.0	7.6	2.5
1	24	20	32	15	15	21	7.1
1½	24	22	29	18	21	23	4.1
2½	19	22	23	14	19	19	3.5
5	13	10	14	10	13	12	1.9
8	7.0	6.5	8.8	4.9	7.6	7.0	1.4
12¼	3.8	3.7	3.3	1.9	3.5	3.2	0.8

was 66 ng/ml. The time required to reach maximum drug levels was 1.3 ± 0.3 h compared to 0.9 h in the dog. After maximum levels were attained the drug disappeared from serum with a first-order rate, and the half-life of this portion of the curve was 3.8 ± 0.3 h. There was no prominent II peak in human serum extracts (Fig. 2), suggesting that the drug may be metabolized to a lesser degree or by different routes than in the dog.

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