Quantitative GLC Determination of Oxycodone in Human Plasma

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Abstract □ A GLC assay was developed for the determination of oxycodone levels in human plasma using a nitrogen-specific detector. The assay was developed for use in bioavailability studies of therapeutic doses of oxycodone. After ingestion of a commercial tablet containing 4.5 mg of oxycodone hydrochloride and 0.38 mg of oxycodone terephthalate by six volunteers, the mean peak oxycodone concentration in plasma was 18.4 ng/ml at 1 hr.

Keyphrases □ Oxycodone—GLC analysis in plasma □ GLC—analysis, oxycodone in plasma □ Narcotic analgesics—oxycodone, GLC analysis in plasma

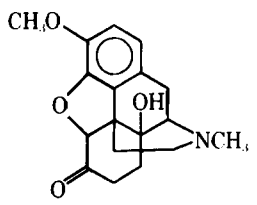
Oxycodone (14-hydroxydihydrocodeinone) (I) is a narcotic analgesic (1) and antitussive agent (2). For many years, the detection and determination of oxycodone were based largely on TLC and UV spectrophotometry (3–5). Recently, a GLC procedure for the quantitation of oxycodone hydrochloride in pharmaceutical preparations was reported (6).

However, methods of determining oxycodone levels in human plasma have not been available. This problem is inevitably coupled with the fact that the circulating oxycodone in blood from normal subjects after a single therapeutic dose is extremely low. The existing spectrophotometric and GLC methods are not suitable since the lower limit of detection is approximately 1 μg.

This report describes a GLC assay for oxycodone [with corydine hydrochloride (II) as the internal standard] in plasma using a nitrogen-specific detector. The assay requires 5 ml of plasma and is capable of determining 2 ng of oxycodone/ml.

EXPERIMENTAL

Materials—Nanograde quality methanol and chloroform[1] were used without further purification. Thirty-milliliter glass bottles[2] with polyethylene screw caps[3] were used for the plasma extraction, and 12-ml glass-stoppered centrifuge tubes[4] were used for the final solvent evaporation. Volumetric glass pipets were utilized for all solvent transfers. All glassware was soaked in chromic acid for at least 1 hr, rinsed thoroughly with distilled water, and heat treated for 3 hr at 270°. Polyethylene screw caps were soaked in *n*-heptane for at least 1 hr and dried at 60° prior to use.



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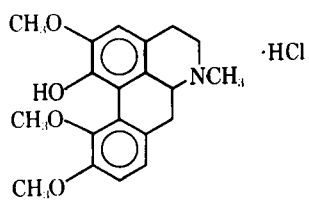

II

Stock solutions of oxycodone and corydine (1-hydroxy-2,10,11-trimethoxyaporphine), an internal standard, were prepared in chloroform using oxycodone hydrochloride[1] and corydine hydrochloride[5] with all concentrations expressed in terms of the free base. Both compounds showed satisfactory UV, IR, NMR, and TLC properties. Clinical control plasma[6] was used in the preparation of the oxycodone plasma standards.

Apparatus—A gas chromatograph[7] equipped with a nitrogen–phosphorus-specific detector was used. The GLC column was a 0.91-m × 2-mm i.d. silanized glass column packed with 2% OV-101 on 100–120-mesh Chromosorb WHP[8]. The column was conditioned at 270° overnight with a helium flow of 30 ml/min.

Chromatographic temperatures were: column oven, 240°; injection port, 260°; and detector, 300°. The helium carrier gas flow rate was 30 ml/min, the hydrogen flow rate was 3 ml/min, and the air flow rate was 100 ml/min.

For the positive identification of oxycodone in human plasma, a quadrupole mass spectrometer[9] and a data system[10] were used in conjunction with the gas chromatograph[11]. A 1.51-m × 2-mm i.d. silanized glass column packed with 3% OV-1 on 60–80-mesh Gas Chrom Q[12] was conditioned at 270° overnight with a helium carrier gas flow rate of 20 ml/min. During analysis, the interface oven and the transfer line in the GLC–mass spectrometry system were at 250°. The column temperature was 235°, and the injection port temperature was 280°.

The methane[13] pressure was adjusted to provide an ion source pressure

Figure 1—*Typical gas chromatogram of a plasma sample seeded with 10 ng of oxycodone/ml of plasma.*

[1] Mallinckrodt Chemicals.
[2] Wheaton Co.
[3] Standard Cap Corp.
[4] Arthur H. Thomas Co.
[5] Eastman Organic Chemicals.
[6] Interstate Blood Bank, Philadelphia, Pa.
[7] Hewlett-Packard model 5710A.
[8] Hewlett-Packard.
[9] Finnigan model 3300.
[10] Finnigan model 6110.
[11] Finnigan model 9500.
[12] Applied Science Laboratories.
[13] Instrument purity, Matheson.

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Table I—Means, Standard Deviations, and Coefficients of Variation of Area Ratios at Various Oxycodone Concentrations

Oxycodone Concentration, ng/ml	Number of Determinations	Area Ratios		
		Mean	*SD*	*CV*
2	5	0.163	0.49	0.30
5	10	0.257	0.061	0.23
10	9	0.517	0.111	0.21
20	9	1.084	0.171	0.16
30	9	1.608	0.277	0.17
50	5	2.802	0.224	0.08

of 1 torr. The ion source settings were: ion energy, 2 v; ion repeller, 0 v (chemical ionization), 35 v (electron impact); ion lens, 40 v; and filament, 70 v (electron impact), 150 v (chemical ionization). The electron beam total emission was regulated at 0.55 mamp, while the source was operated without external heating.

Assay Procedure—Five milliliters of plasma, 10.0 ml of internal standard in chloroform solution (20 ng of corydine/ml), and 1.0 ml of 1 *N* NaOH were added to a 30-ml bottle, and the mixture was shaken on a tabletop shaker[14] at 120 oscillations/min for 15 min. After centrifugation[15] at 2000 rpm, the aqueous phase was removed and discarded. To the organic phase was added 5.5 ml of 0.05 *N* HCl. The mixture was then shaken for 15 min and centrifuged for 10 min.

Five milliliters of the aqueous layer was transferred to a 30-ml bottle containing 10.0 ml of chloroform and 1.0 ml of 1 *N* NaOH. The mixture was shaken for 15 min and centrifuged for 10 min, and the aqueous phase was removed and discarded. Then 8 ml of the organic phase was pipetted into a 12-ml centrifuge tube, and the solvent was evaporated under a nitrogen stream at 40°. The residue was reconstituted in 20 μl of methanol, and 10 μl was injected into the gas chromatograph.

Oxycodone Single-Dose Human Plasma Samples—Six healthy adult male volunteers were fasted for at least 12 hr prior to dosing and 1 hr postdosing. Each subject was dosed with a single tablet[16] and 200 ml of distilled water. Blood samples of 20 ml were collected in heparinized vacuum tubes by venipuncture at 0 (predose), 10, 20, 40, 60, and 90 min and 2, 3, 4, 5, 6, and 8 hr after dosing. The plasma was separated by centrifugation immediately after collection, and the clear plasma was stored frozen at −10° in polypropylene snap-cap tubes until assayed (within several weeks).

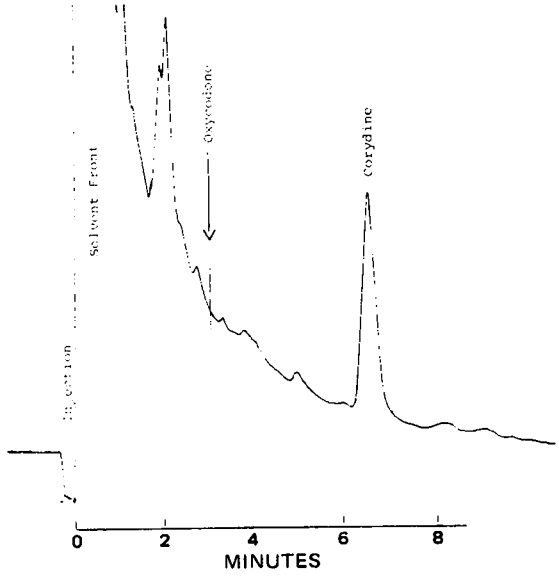

Figure 2—*Typical gas chromatogram of a blank plasma sample containing corydine as an internal standard.*

[14] Eberbach Corp.
[15] International Centrifuge.
[16] Percodan, Endo Laboratories, Inc. Each tablet contained 4.5 mg of oxycodone hydrochloride, 0.38 mg of oxycodone terephthalate, 224 mg of aspirin, 160 mg of phenacetin, and 32 mg of caffeine.

Table II—Plasma Oxycodone Levels (Nanograms per Milliliter) in Six Subjects after Oral Administration of 4.5 mg of Oxycodone Hydrochloride and 0.38 mg of Oxycodone Terephthalate

Time after Administration	Subject						
	1	2	3	4	5	6	Mean
0	0	—	0	0	0	0	0
10 min	0	0.8	0	0	0	0	0.1
20 min	6.3	2.0	0	5.5	1.8	0	2.6
40 min	13.0	19.8	6.3	21.0	13.0	5.7	13.1
60 min	36.5	12.9	16.5	20.0	16.0	8.5	18.4
90 min	12.5	22.8	19.5	15.3	25.0	8.7	17.3
2 hr	10.7	18.9	17.0	12.3	18.3	17.0	15.7
3 hr	10.0	11.5	12.3	7.0	13.8	6.0	10.1
4 hr	8.0	8.8	10.7	9.6	11.9	7.3	9.4
5 hr	6.0	7.0	9.9	9.0	11.3	4.3	7.9
6 hr	3.7	6.3	5.5	5.0	10.0	1.7	5.4
8 hr	3.0	4.0	2.3	3.5	14.3	1.0	4.7

RESULTS AND DISCUSSION

The plasma level profiles of oxycodone in humans have not been reported, undoubtedly because of the difficulties in measuring low levels of oxycodone after a single oral therapeutic dose. The TLC and UV spectrophotometric assays (3–5) as well as the GLC method, using flame-ionization detection (6), do not offer enough sensitivity for determining oxycodone levels in human plasma samples.

Due to the lack of an electron-capturing moiety in the oxycodone molecule, the electron-capture detector has no better sensitivity than the flame-ionization detector toward oxycodone. Derivatization of oxycodone with acetic anhydride and propionic anhydride was unsuccessful (5). Other available derivatization reagents for electron-capture detection appeared not to react with oxycodone. The nitrogen-phosphorus-specific detector used in the nitrogen mode, however, shows good sensitivity in detecting oxycodone. The lower limit of detection is 2 ng or better, and the detector response is linear between 2 and 50 ng.

Oxycodone is a weak base with a pKa of 8.53 (7); the compound can be extracted from plasma into an organic solvent, such as chloroform, in the presence of a strong base. Oxycodone forms salts with strong acids such as hydrochloric, and these salts are readily soluble in water. With the present extraction procedure, the mean extraction efficiencies ± *SD* for 10 determinations were 72.8 ± 10.8% for oxycodone at concentrations of 10 ng/ml and 71.6 ± 15.3% at 20 ng/ml. Corydine had a mean extraction efficiency of 54.1 ± 6.3% (n = 6) at 20 ng/ml (concentration used in the assay).

Figure 1, a typical oxycodone chromatogram tracing, shows the separation of oxycodone and corydine peaks. The retention times under the experimental conditions were 3.3 and 6.9 min for oxycodone and corydine, respectively. Shown in Fig. 2 is the chromatogram tracing of a blank plasma extract. No plasma peaks interfered with the assay.

The peak area ratios of replicate samples (expressed as oxycodone to corydine), obtained from the addition of known amounts of oxycodone to control blank plasma followed by processing according to the described procedure, are summarized in Table I. A least-squares analysis gave a straight-line standard curve with a correlation coefficient of 0.9992, a slope of 0.056 ± 0.01, and an intercept of −0.010 ± 0.029, which, within experimental error, showed that the line passed through the origin. The assay is reproducible, as shown by coefficients of variation in Table I.

In the assay development, the hydrochloride salt of oxycodone was used to prepare oxycodone standards. However, the hydrochloride salt is hygroscopic, and the terephthalate salt is a better compound for this purpose[17].

The identity of the GLC peak was confirmed by GLC–mass spectrometry using both electron-impact and chemical-ionization modes of analysis. The electron-impact spectrum exhibited a molecular ion at m/e 316 for the component in the GLC peak, which is identical to the spectrum of authentic oxycodone obtained by the direct inlet technique. The chemical-ionization spectrum showed mass peaks at m/e 316 (M + 1), 344 (M + 29), and 356 (M + 41), which are characteristic peaks in the chemical-ionization spectrum of oxycodone with methane as a reagent gas. Furthermore, the chemical-ionization spectrum of the component in the GLC peak was also identical to that of authentic oxycodone by the direct inlet method.

[17] Pharmacy Research Department, McNeil Laboratories, unpublished results.

Since the metabolism of oxycodone has not been fully established, the GLC characteristics of the metabolite(s) are yet unknown. However, from the expected metabolic pathways of iminoethanophenanthrofurans (8), it may be speculated that the metabolites have different retention in the GLC column than oxycodone under the same chromatographic conditions. Drugs usually coadministered with oxycodone such as acetaminophen, aspirin, caffeine, and phenacetin do not interfere in the assay. Without derivatization, acetaminophen and aspirin do not pass through the GLC column while phenacetin and caffeine appear to be eluted with the solvent.

The results of single-dose administrations of 4.5 mg of oxycodone hydrochloride and 0.38 mg of oxycodone terephthalate to human subjects are listed in Table II. The maximum plasma oxycodone levels ranged from 17.0 to 36.5 ng/ml in the six subjects. After 8 hr, the oxycodone levels ranged from 1.0 to 14.3 ng/ml.

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Interaction of the Antimalarial α-Dibutylaminomethyl-2,6-bis(trifluoromethylphenyl)-4-pyridinemethanol with Human Serum Albumin

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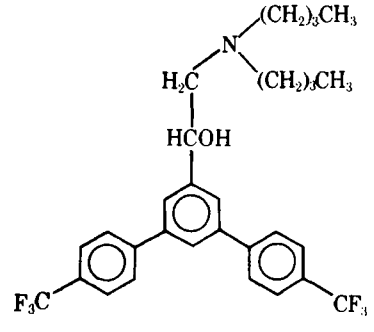
Abstract □ Binding of the antimalarial α-dibutylaminomethyl-2,6-bis(trifluoromethylphenyl)-4-pyridinemethanol with human serum albumin was studied using difference spectroscopy, fluorescence quenching, and equilibrium dialysis. Results indicated that the number of high affinity binding sites of the drug on protein is 0.45, with the total number of binding sites being 3.3–4.0. The binding constants were in the range of 0.57–4.00 × 10^6 M^{-1}. The drug was bound more strongly to a nonionic detergent than to either a cationic or anionic detergent. Interpretation of these data and fluorescence quenching results indicated that the drug is possibly bound to a hydrophobic site on human serum albumin.

Keyphrases □ Pyridinemethanol, substituted—binding to human serum albumin □ Binding, protein—substituted pyridinemethanol to human serum albumin □ Antimalarials—α-dibutylaminomethyl-2,6-bis(trifluoromethylphenyl)-4-pyridinemethanol, binding to human serum albumin □ α-Dibutylaminomethyl-2,6-bis(trifluoromethylphenyl)-4-pyridinemethanol—binding to human serum albumin

Interest in protein binding of drugs led to an investigation of the antimalarial α-dibutylaminomethyl-2,6-bis(trifluoromethylphenyl)-4-pyridinemethanol[1] (I). The drug is 22 times more effective than quinine and is active against highly chloroquine-resistant *Plasmodium berghei* in mice[2]. There is a lack of information about binding of such pyridinemethanols to serum proteins. Such data are important to an understanding of the distribution, metabolism, and excretion of these antimalarials since only the fraction of drug unbound exerts chemotherapeutic activity (1, 2).

The hydrophobic alkyl side chains, the aromatic π-electrons, the hydrogen-bonding site (OH group), and the possible electrostatic interactions suggest that the drug



I

may have a high affinity for serum albumin (3). The limited water solubility of the drug, even as the hydrochloride salt, makes the examination of binding affinity difficult. In this paper, the interaction of I with human serum albumin is examined by difference spectrophotometry, fluorescence quenching, and equilibrium dialysis.

EXPERIMENTAL

Apparatus and Chemicals—Compound I was supplied as the hydrochloride salt[3]. Its purity was verified by TLC in three different solvent systems (4) and by mass spectral analysis. Human serum albumin[4] was investigated for purity (5). The $E^{1\%}_{1cm}$ values for the albumin were 6.36 at 279 nm and 190 at 210 nm. Sorensen phosphate buffer, adjusted to pH 7.4, was used.

The dialysis membrane was prepared according to literature methods (6–8). Rigid, clear acrylic plastic dialysis cells (10 ml) were washed repeatedly with distilled water and sonicated overnight in distilled water. Electronic absorption spectra[5] were taken in 1-cm silica cells. Fluores-

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[3] Walter Reed Army Institute of Research, Washington, D.C.

[4] Armour and Co.

[5] Cary spectrophotometer, model 118, Varian Instrument, Palo Alto, Calif.

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