Determination of Oxycodone in Plasma and Identification of a Major Metabolite

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Abstract □ An electron-capture GLC method is described for oxycodone and its major metabolite, noroxycodone, in plasma and urine. The method involves extraction of the two substances into benzene–isopropanol at pH 10.4, followed by back-extraction into 0.1 *N* HCl. The acid phase is washed with hexane and made alkaline prior to reextraction into benzene–isopropanol. The solvent is removed by evaporation, and the heptafluorobutyryl derivatives of the test substances are formed. After removal of excess reagent, oxycodone and noroxycodone are quantitated by GLC. The characteristics of both substances, with respect to plasma levels in dogs and analgesic activity in mice, are reported. Isolation of noroxycodone from human urine and its identification by TLC, GLC, and mass spectrometry are described.

Keyphrases □ Oxycodone—analysis in plasma and urine, metabolites, electron-capture GLC method □ GLC, electron capture—analysis, oxycodone and noroxycodone in plasma and urine □ Noroxycodone—analysis in plasma and urine, electron-capture GLC method

Oxycodone (4,5α-epoxy-14-hydroxy-3-methoxy-17-methylmorphinan-6-one) has pharmacological properties similar to those of codeine and is used in combination with aspirin, phenacetin, and caffeine[1] and with acetaminophen[2] in the treatment of moderate to moderately severe pain (1). Existing methods (2, 3) for the determination of oxycodone in biological materials, while useful as screening procedures for forensic purposes, are not sufficiently sensitive to determine the drug in plasma after clinical doses.

This report describes a specific, sensitive analytical method for plasma oxycodone and noroxycodone, its major metabolite.

EXPERIMENTAL

Extraction of Oxycodone and Noroxycodone from Plasma—A 2-ml plasma sample containing oxycodone and noroxycodone was placed in a 25-ml screw-capped centrifuge tube[3], and 25 ng of naltrexone hydrochloride[4] (in 0.1 ml of aqueous solution), the internal standard, was added followed by 1 ml of distilled water. The pH was adjusted to 10 with 1 *N* NaOH, and 1.5 ml of pH 10.4 phosphate buffer[5] was added, followed by 1.5 g of sodium chloride.

This mixture was extracted with 10 ml of benzene[6] containing 1% (v/v) isopropanol by shaking on a wrist-action shaker for 30 min. After centrifugation for 15 min at 2200×*g*, 9 ml of the upper organic layer was transferred into a 15-ml glass-stoppered centrifuge tube containing 1 ml of 0.1 *N* HCl. The tube was agitated on a mixer[7] for 1 min and then centrifuged for 10 min at 1240×*g*.

The upper organic phase was discarded, and the remaining aqueous layer was washed with 5 ml of hexane[6]. After the hexane was discarded, the aqueous phase was adjusted to pH 10 with 0.1 *N* NaOH, and 1.5 ml of pH 10.4 phosphate buffer was added. The alkaline aqueous phase was extracted into 6 ml of benzene containing 1% isopropanol by shaking for 30 min on a wrist-action shaker. After centrifugation at 1240×*g* for 10 min, 5 ml of the upper benzene phase was transferred, 2.5 ml at a time, to a 5-ml glass-stoppered centrifuge tube and evaporated to dryness[8].

Preparation of Plasma Standards—Aqueous solutions of oxycodone hydrochloride in the concentration range from 100 to 6.25 ng/ml were prepared by serial dilution. Aliquots of these solutions were added to control plasma samples such that the concentrations of oxycodone hydrochloride in plasma ranged from 10.0 to 0.625 ng/ml. The internal standard was added, and extraction and quantitation were performed as already described.

Expired human plasma obtained from blood banks and dog plasma were both used for oxycodone plasma standards.

Derivatization and GLC—The heptafluorobutyryl derivatives of oxycodone, noroxycodone, and naltrexone (the internal standard) were prepared by treating the dried residue of the extract with heptafluorobutyrylimidazole[9] (I). Ten microliters each of benzene and I were added to the dried residue. The mixture was agitated on a mixer for 3 sec and incubated at 85° for 45 min in a heating block[10]. After cooling, 50 μl of toluene was added to the reaction mixture. The toluene solution was washed once with 1 ml of 2.5% aqueous NH_4OH and then three times with distilled water. After each washing, the mixture was centrifuged and the lower aqueous phase was removed with a Pasteur pipet.

A 2- or 4-μl aliquot of the toluene solution was injected into the gas chromatograph[11], which was equipped with a 15-mCi ^3H-electron-capture detector. The 1.8-m glass column, packed with 3.8% methyl phenyl silicon gum rubber[12], had been previously conditioned for 48 hr at 250° with a helium flow rate of 12 ml/min. The oven, detector, and injection port temperatures were 215, 220, and 220°, respectively. The pulse interval setting was 15. The carrier was helium, 75 ml/min; 10% methane in argon, 100 ml/min, was the purge gas.

The peak height ratios of oxycodone and naltrexone heptafluorobutyrates and of noroxycodone and naltrexone heptafluorobutyrates were determined and were proportional to the amounts of oxycodone and noroxycodone injected.

Extraction of Oxycodone and Noroxycodone from Urine—Urine samples were extracted for oxycodone or noroxycodone analysis by diluting 0.1 ml of urine with 2.9 ml of distilled water. The pH of the diluted urine was adjusted to 1.0 with 5 *N* H_2SO_4. The diluted, acidified urine was washed with 10 ml of benzene on a mixer for 1–2 min. Then the benzene wash was discarded, and the aqueous phase pH was adjusted to 10 with 5 *N* NaOH. The extraction was then performed as already described for plasma.

After formation of the heptafluorobutyryl derivatives, the samples were reconstituted in 100 μl of toluene and washed as described for plasma extracts. Then 1-μl aliquots were injected. The oxycodone or noroxycodone concentration in urine was determined using an external standard consisting of control urine with known amounts of oxycodone added. Control urine samples with oxycodone hydrochloride added to yield concentrations between 100 and 2500 ng/ml were appropriate standards. The internal standard used in plasma was obscured by an interfering peak in urine extracts.

Metabolite Isolation and Identification—For TLC and subsequent mass spectrometry, 10 ml of urine containing oxycodone and noroxycodone (spiked control urine containing 10 μg/ml of each was used as a standard) was adjusted to pH 1.0 with 5 *N* H_2SO_4. The acidified urine was washed with benzene and readjusted to pH 10 with 5 *N* NaOH. After addition of 1.5 ml of pH 10.4 phosphate buffer and 2 g of sodium chloride, the urine sample was extracted with 6.0 ml of benzene. Then the benzene extract was evaporated to dryness.

[1] Percodan, Endo Laboratories.
[2] Percocet-5, Endo Laboratories.
[3] Corex tube with Teflon-lined cap.
[4] Naltrexone hydrochloride, oxycodone hydrochloride, and noroxycodone hydrochloride were obtained from Endo Laboratories.
[5] Potassium phosphate buffer is prepared by adding 5% aqueous K_3PO_4-H_2O to 35% aqueous K_2HPO_4 until pH 10.4 is attained. Both phosphate salts are "Baker analyzed" reagent grade.
[6] All organic solvents used are Burdick & Jackson, distilled in glass.
[7] Vortex.
[8] Evaporation was performed with a Buchler Evapo-Mix attached to a water aspirator.
[9] Pierce Chemicals, Rockford, Ill.
[10] Temp-Blok, Lab Line Instruments, Melrose Park, Ill.
[11] Hewlett-Packard model 7610A.
[12] OV-17 on 80–100-mesh Gas Chrom Q.

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Table I—Plasma Concentrations (Nanograms per Milliliter) of Oxycodone and Noroxycodone in Dogs

Minutes	Dog 1, 0.5 mg/kg po		Dog 2, 0.1 mg/kg po	
	Oxycodone	Noroxycodone[a]	Oxycodone	Noroxycodone[a]
0	ND[b]	ND	ND	ND
5	3.0	11.5	...[c]	—[c]
7	—[c]	—[c]	ND	ND
15	6.5	34.5	1.1	1.7
30	5.8	34.7	2.3	7.5
45	5.3	38.3	4.4	—[d]
60	3.8	28.4	2.6	4.2
90	2.7	31.5	1.6	3.2
120	4.8	23.7	1.5	7.4
180	2.1	17.5	0.8	7.0
240	ND	21.3	ND	4.9
420	ND	14.7	—[c]	—[c]
24 hr	ND	ND	—[c]	—[c]

[a] Calculated as oxycodone equivalents. [b] ND = not detectable. [c] No sample. [d] Quantitation impossible due to interfering peaks.

Table II—Effects of Oxycodone Hydrochloride and Noroxycodone Hydrochloride in Mouse Phenylquinone Writhing Test

Route	ED_{50}[a], mg/kg	
	Oxycodone	Noroxycodone
Oral	0.74	26
Subcutaneous	0.16	22

[a] See text for details.

The residue was reconstituted with 100 μl of methanol, and 20 μl was spotted on a 0.25-mm silica gel thin-layer plate[13]. The developing solvent was chloroform–ethanol (absolute)–concentrated ammonium hydroxide (50:50:1 v/v). Oxycodone and its metabolite were detected with Dragendorf spray reagent oversprayed with 5% aqueous $NaNO_2$ (4).

The area of the thin-layer plate corresponding to the R_f of standard noroxycodone was scraped from the plate, and the metabolite was eluted from the silica with 1 ml of methanol. Then the eluate was evaporated to dryness.

The mass spectrum of the isolated metabolite in the dried residue was determined with a high-resolution mass spectrometer[14]. The mass spectrum of noroxycodone was also determined for comparison.

Animal Studies—One male beagle dog was given oxycodone hydrochloride by gavage, 0.5 mg/kg in 100 ml of water. Blood samples were collected from the jugular vein into heparinized tubes[15] at 0, 5, 15, 30, 45, 60, 90, 120, 180, and 240 min and 24 hr after dosing.

To determine plasma concentrations at a dose approximating the clinical dose, another male beagle dog was given, by gavage, 0.1 mg of oxycodone hydrochloride/kg in 100 ml of water. Blood samples were collected as already described at 0, 7, 15, 30, 45, 60, 90, 120, 180, and 240 min.

Plasma was extracted and derivatized, and oxycodone and noroxycodone concentrations were determined as already described. Noroxycodone is reported quantitatively as oxycodone equivalents.

Oral and parenteral activities of the metabolite were compared with that of the parent compound in a modification of the mouse phenylquinone writhing test described by Siegmund *et al.* (5) Twenty mice were used for oxycodone evaluations, and 10 were used for noroxycodone. Noroxycodone values were determined at the peak time observed for oxycodone by the oral (20 min) and subcutaneous (5 min) routes.

Metabolite Identification—A urine sample obtained from a patient being treated with high doses of oxycodone by his personal physician (*i.e.*, approximately 700 mg/day) was processed as already described for isolation and identification of the metabolite. The urine extract was subjected to analysis by GLC, TLC, and mass spectrometry.

[13] Brinkmann silica gel 60.
[14] Du Pont model 21-492.
[15] Vacutainer.

RESULTS

GLC and Extractions—Under the described chromatographic conditions, the heptafluorobutyryl derivatives of oxycodone and naltrexone had retention times of 3.5 and 5.7 min, respectively. There were no interfering peaks from either human or dog plasma extracts; however, an interfering peak from urine prevented the use of naltrexone with urine samples. The recovery of oxycodone was 90% of an unextracted standard. There was a linear, reproducible relationship betwen the electron-capture GLC response and the plasma or urine oxycodone concentration.

Reproducibility of peak height ratios for quadruplicate samples with oxycodone hydrochloride added to contain 10.0 and 1.0 ng/ml was 6.0 and 17.0% (percent standard deviation), respectively.

When urine and plasma samples were analyzed by this method, a peak with a retention time of 4.8 min was consistently observed. This peak did not appear in any zero-time, blank, or control samples, and evidence is presented here for its identity with noroxycodone.

Metabolite Identification—The heptafluorobutyryl derivative of noroxycodone was prepared under the same conditions used for dog plasma and human urine analysis. The derivative of noroxycodone had a retention time of 4.8 min when subjected to the GLC conditions identical to those used for the metabolite.

TLC yielded R_f values of 0.15 for both the metabolite isolated from human urine and standard noroxycodone and 0.73 for oxycodone. The concentrations, determined by GLC, of oxycodone and noroxycodone in the human urine sample were 12.9 and 18.3 μg/ml, respectively.

Mass spectral data confirmed that the metabolite isolated by TLC from human urine was noroxycodone. The mass spectrum showed a strong molecular ion peak at m/e 301 and a characteristic fragment pattern that agreed well with the pattern obtained for the standard noroxycodone.

Animal Studies—In dogs given 0.1 and 0.5 mg/kg of oxycodone po, plasma noroxycodone levels were higher than those of the parent compound (Table I). In mice, results of the phenylquinone writhing test (Table II) indicated that noroxycodone is considerably less active than oxycodone, both orally and parenterally. Moreover, in contrast to observations of mice given oxycodone, the Straub tail response was not observed following noroxycodone administration.

REFERENCES

(1) "AMA Drug Evaluations," 2nd ed., Publishing Sciences Group, Acton, Mass., 1973, p. 272.
(2) S. J. Mulé, *Anal. Chem.*, **36**, 1907 (1964).
(3) S. J. Mulé, *J. Chromatogr. Sci.*, **10**, 275 (1972).
(4) W. W. Fike, *Anal. Chem.*, **38**, 1967 (1966).
(5) E. Siegmund, R. Cadmus, and G. Lee, *Proc. Soc. Exp. Biol. Med.*, **95**, 729 (1957).

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