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Nanogram level quantitation of oxycodone in human plasma by capillary gas chromatography using nitrogen–phosphorus selective detection

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ABSTRACT

A sensitive and specific capillary gas chromatographic assay is reported for the quantitation of oxycodone in human plasma. The technique involves a single extraction of oxycodone and internal standard (hydrocodone) from plasma by toluene containing 1% isopropanol. Separation is achieved on a methyl silicone (HP-1) fused-silica capillary column (25 m × 0.2 mm I.D., 0.33 μm film thickness) and detection is by nitrogen–phosphorus selective mode. The minimum quantifiable limit is 1.8 ng/ml using 2 ml of plasma. The method is applicable to characterize the plasma profile of oxycodone in humans after a single oral 5-mg oxycodone hydrochloride tablet.

INTRODUCTION

Oxycodone (4,5-epoxy-14-hydroxy-3-methoxy-17-methylmorphinan-6-one), a semisynthetic narcotic analgesic (Fig. 1), has been in clinical use worldwide for the past seventy years. It is a potent analogue of codeine [1]. The oral bioavailability of oxycodone has been reported to be better than that of morphine and hence 25% less dosage is required for equal analgesia in cancer patients [2]. The pharmacokinetics of oxycodone in humans have not been adequately characterized despite its clinical use over a long time span. The lack of database might be in part related to the limitation in quantitation of low plasma concentrations of oxycodone after a single oral dose of oxycodone. A recent investigation reported that the maximum plasma oxycodone concentration of 17 ng/ml was achieved following an oral dose of 10 mg oxycodone hydrochloride [3].

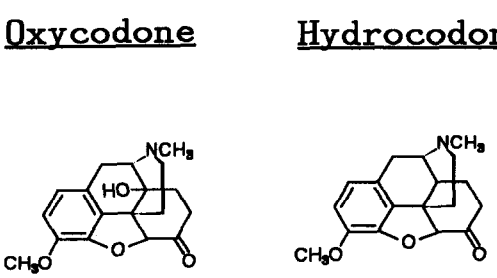

Fig. 1. Structures of oxycodone and hydrocodone.

Several chromatographic methods for quantitation of oxycodone in human plasma have been reported. However, they all have practical limitations such as requiring large sample volume, forming unstable and multiple derivatives and poor reproducibility of the assay. A packed column gas chromatographic (GC) method using nitrogen–phosphorus detector (NPD) has been described [4] to quantify 2 ng/ml oxycodone using 5 ml of plasma. A lengthy chemical derivatization procedure has also been reported using packed

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column GC–electron–capture detection [5]. Exploratory work conducted in our laboratory, however, showed that the derivatization procedure resulted in multiple as well as unstable derivatives. Schneider *et al.* [6] developed a high-performance liquid chromatographic (HPLC) assay using electrochemical detection (ED). The method was irreproducible because of poor resolution of the chromatographic peaks and excessive noise. Recently, Smith *et al.* [3] reported an HPLC–ED assay for oxycodone with a low quantifiable limit of 10 ng/ml. A capillary GC–NPD method was reported by Saarialho-Kere *et al.* [7]. The authors claimed minimum sensitivity of 3 ng/ml based on a peak/baseline ratio of 3 using 1 ml of plasma. However, no quantitative chromatographic details were provided.

This report describes a specific and sensitive fused-silica capillary GC–NPD assay for oxycodone in human plasma using hydrocodone as internal standard. The assay required 2 ml of plasma and the minimum quantifiable limit was 1.8 ng/ml oxycodone base. It was suitable for multiple automatic injection from the same sample as only 2 out of 20 μl of the final reconstituted volume were required per analysis.

EXPERIMENTAL

Chemicals and solutions

Toluene, isopropanol and methanol were chromatographic grade (J. T. Baker, Phillipsburg, NJ, USA). Sodium hydroxide pellets were reagent grade (J. T. Baker). Blank plasma, obtained from human volunteers, was purchased from Biological Specialties (Lansdale, PA, USA). Oxycodone hydrochloride and hydrocodone bitartrate (internal standard, I.S.) were supplied by Du Pont Merck Pharmaceutical (Wilmington, DE, USA).

Instrumentation

A gas chromatograph (Model 5890A, Hewlett Packard, Avondale, PA, USA) equipped with a nitrogen–phosphorus detector, a split–splitless capillary inlet system and an autosampler (Model HP7673A) was used for the analyses. An HP-1 fused-silica capillary column (25 m × 0.2 mm I.D., 0.33 μm film thickness) was utilized for separation of the compounds. A PC-based data acquisition system (Chemstation, Model HP3365, Hewlett Packard) was used to acquire data and for peak integration.

Chromatographic conditions

The splitless injection mode employing a silanized fused-silica inset (78 mm × 2 mm I.D.) with a purge-off time of 1.5 min was used for 2-μl samples. The operating conditions for routine analysis were: injection port temperature, 225°C; initial oven (column) temperature, 150°C; initial hold time, 0 min; programming rate, 35°C/min; final oven temperature, 245°C; NPD temperature, 250°C. The helium flow-rates were: 1.5 ml/min at 40°C and 1.9 bar, and 0.5 ml/min at 150°C and 1.9 bar as carrier gas; 80 ml/min as total vent; 3 ml/min as septum purge; and 15 ml/min as make-up gas. The flow-rates of hydrogen and air for NPD were 3.6 ml/min (at 1.2 bar) and 100 ml/min (at 2.7 bar), respectively.

Stock solutions

Primary stock solutions (100 μg/ml) of oxycodone hydrochloride and hydrocodone bitartrate (I.S.) were prepared by dissolving 10.0 mg of each in distilled water in separate 100-ml volumetric flasks. Subsequent stock solutions of 1 μg/ml and 100 ng/ml were prepared, and aliquots were added to plasma as standards. All aqueous solutions were stored at 4°C and prepared fresh each month.

Preparation of plasma standards and samples, and extraction procedure

Oxycodone plasma standards were prepared by adding 0.04, 0.08, 0.16, 0.24 and 0.4 ml of working stock solution of 100 ng/ml oxycodone hydrochloride into 15-ml glass tubes (16 × 125 mm) containing 2 ml of blank human plasma. The volume in each tube was adjusted to 3 ml with distilled water. The resultant plasma concentrations of oxycodone base were 1.79, 3.59, 7.17, 10.76 and 17.93 ng/ml. To each tube, the following components were added: 0.4 ml of 100

ng/ml I.S., 0.75 ml of 0.5 *M* NaOH and 6 ml of extraction solvent, isopropanol–toluene (1:99, v/v). The preparation of plasma samples was similar to that of standards with the exception of following changes: (a) 1 ml of distilled water was used in place of oxycodone hydrochloride stock solution; (b) 2 ml of blank plasma was replaced with 2 ml of plasma sample. Plasma standards and samples were extracted twice by the organic extraction solution (2 × 6 ml) by shaking for 5 min on a modified Labquake rocking shaker. The tubes were then centrifuged (Sorval RC-3B equipped with an H-6000 swinging bucket rotor, Du Pont Instruments, Wilmington, DE, USA) for 5 min at 2620 *g*. If an emulsion was formed after centrifugation, the tubes were shaken manually by hand for 1 min and recentrifuged for 5 min. A 5-ml volume of the organic phase from each extraction was combined in silanized 15-ml clean glass centrifuge tubes. It was evaporated to dryness on a 40°C water bath (N-EVAP, Organomation, Berlin, MA, USA) under a gentle stream of nitrogen. Methanol (0.5 ml) was added to each tube followed by vortex-mixing for 10 s to dissolve any residue from the sides of the tubes. After the evaporation of methanol under nitrogen on a 40°C water bath, the residue was reconstituted with 20 μl of toluene containing 1% isopropanol and vortex-mixing for 10 s. The contents of each tube was transferred to injection vials containing silanized inserts. Of each sample, 2 μl were injected onto the gas chromatograph.

Calculations and data analysis

Integration of oxycodone and I.S. peaks were performed by a PC-based data acquisition system. Peak-height ratio (PHR) of oxycodone/internal standard for each sample was determined. Linear regression analysis of PHRs of plasma standard *versus* oxycodone concentrations were calculated. The concentrations of unknown samples were calculated by interpolation from the linear regression equation.

All plasma concentrations are expressed as the free base of oxydocone. Some calculations were performed using the peak-area ratios instead of PHRs if required by the chromatographic results.

Human pharmacokinetics

Oxycodone hydrochloride (Percocet) was given as a single oral dose (5 mg) to a fasted male healthy volunteer. Blood samples were collected from the brachial vein via an indwelling butterfly cannula at predose and at 20, 40, 60 90, 120, 180, 240, 360 and 420 min in heparinized Vacutainers (Becton-Dickinson, Rutherford, NJ, USA). Plasma was obtained following centrifugation (Sorval 600B) for 15 min at 2122 *g*. Plasma samples were stored at −20°C until analyzed.

RESULTS AND DISCUSSION

Representative chromatograms obtained from the extraction of a human blank plasma, plasma spiked with oxycodone and a plasma sample after oral administration of oxycodone hydrochloride are presented in Fig. 2. No interference from

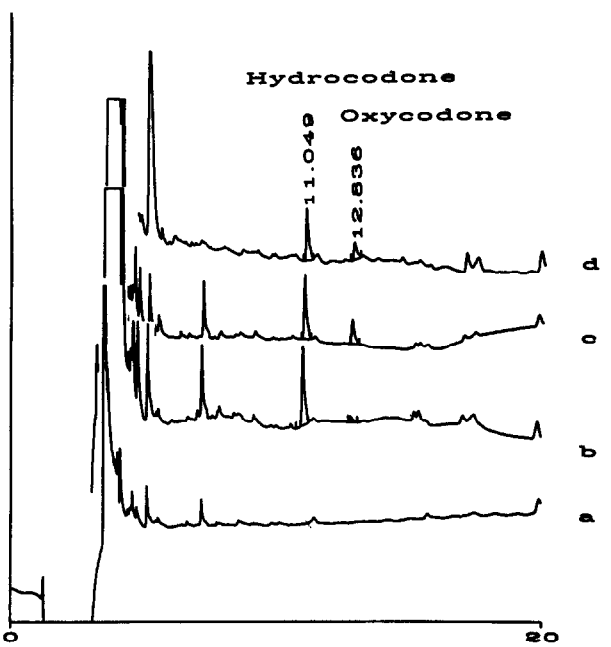

Fig. 2. Representative chromatograms of extracted samples: (a) 2 ml of control human plasma; (b) 2 ml of control human plasma spiked with 40 ng of hydrocodone bitartrate (I.S.) and 4 ng of oxycodone hydrochloride; (c) same as (b) except 24 ng of oxycodone hydrochloride; and (d) 2 ml of human plasma at 90 min after receiving a single 5-mg oral dose of oxycodone hydrochloride. The *x*-axis represents time after injection in min. The retention times for hydrocodone and oxycodone were 11.0 and 12.8 min, respectively.

TABLE I

PRECISION FOR THE QUANTITATION OF OXYCODONE BASE IN HUMAN PLASMA

Values are mean ± S.D., $n = 3$.

Spiked concentration (ng/ml)	Intra-day		Inter-day	
	Found concentration (ng/ml)	C.V. (%)	Found concentration (ng/ml)	C.V. (%)
1.79	1.62 ± 0.17	10.7	1.95 ± 0.16	8.3
3.58	3.68 ± 0.43	11.5	3.59 ± 0.42	11.7
7.16	6.91 ± 0.22	3.2	7.53 ± 0.55	7.3
10.74	11.33 ± 0.36	3.2	10.22 ± 0.60	5.8
17.90	18.88 ± 0.94	5.0	18.60 ± 0.37	2.0

endogenous substances in plasma was detected at the retention times of oxycodone or I.S., hydrocodone, following the extraction procedure. The retention times were 11.0 and 12.8 min for hydrocodone and oxycodone, respectively. The chromatographic peaks of the two compounds were well resolved.

Standard curve for oxycodone assay in human plasma was linear between 1.8 to 17.9 ng/ml. The correlation coefficients of standard curves for the human plasma assay usually exceeded 0.99.

Intra-day reproducibility

Triplicate plasma samples of oxycodone were prepared in the concentration range 1.8–17.9 ng/ml (base equivalent). The samples were extracted and analyzed on the same day. The results are summarized in Table I. The coefficients of variation (C.V.) for the intra-day precision results ranged from 3.2 to 11.5%.

Inter-day reproducibility

Human plasma blanks, spiked with oxycodone in the concentration range 1.8–17.9 ng/ml (base equivalent), were extracted and analyzed on the same day. The results obtained from similar extraction on different occasions are summarized in Table I. The C.V.s for the inter-day precision results ranged form 2.0 to 11.7%.

Accuracy

The results from the accuracy studies in which unknown concentrations of oxycodone in human plasma were quantitated are shown in Table II. The difference between the spiked and found concentrations of oxycodone ranged from −1.8 to 15.0%.

Extraction recovery

The extraction recovery was determined by comparing the peak responses of extracted and unextracted standards of oxycodone at 1.8 and 17.9 ng/ml (base equivalent) concentrations. The extraction recovery of oxycodone from 2 ml of human plasma was 69.4 and 67.5% at concentrations of 1.8 and 18.0 ng/ml, respectively.

TABLE II

ACCURACY OF THE GC-NPD ASSAY FOR OXYCODONE BASE IN HUMAN PLASMA

Spiked concentration (ng/ml)	Found concentration (ng/ml)	Difference (%)
2.68	2.84	5.7
2.68	2.83	5.2
2.68	2.63	−1.8
8.05	9.34	15.0
8.05	8.32	3.0
8.05	7.69	−5.0
14.32	14.68	2.5
14.32	16.25	13.0
14.32	15.18	−2.8

TABLE III

STABILITY OF OXYCODONE AT −20°C IN HUMAN PLASMA

Values are mean ± S.D., $n = 3$.

Spiked concentration (ng/ml)	Stability day	Found concentration (ng/ml)	Difference (%)
1.79	0	1.90 ± 0.17	6.1
	7	2.24 ± 0.59	25.1
	30	2.01 ± 0.02	12.2
17.9	0	18.15 ± 1.42	1.4
	7	16.29 ± 3.2	−8.9
	30	15.95 ± 2.41	−10.9

Stability

The stability of oxycodone in frozen (−20°C) human plasma was determined at concentrations of 1.8 and 17.9 ng/ml. Blank human plasma was spiked with oxycodone hydrochloride and 2-ml aliquots were transferred into individual tubes which remained frozen until assayed on three separate occasions. The results are summarized in Table III. Oxycodone appears to be stable in human plasma, stored frozen at −20°C, for at least 30 days.

Optimization of instrumentation

Routine maintenance of the gas chromatograph including cleaning or replacement of injection port liner and the metal base (HP part No. 18740-20880) was essential for optimal results with respect to chromatographic peak shape and size. These components were either replaced or cleaned after ~200 injections. Injection port septum was replaced after ~50 injections. The column was replaced after ~300 injections.

Consistency in linearity of standard curves required use of silanized glassware during the evaporation step of the assay. Transfer of small volume (20 μl) of reconstituted sample into silanized injection vial inserts was performed using SMI pipettors equipped with silanized glass pipet tips. Drying of samples before injection was avoided by careful crimping of the vial caps and not allowing the sample to sit in the injection tray for too long. The vial caps of samples requiring reinjection at a later time were replaced with new sealed caps.

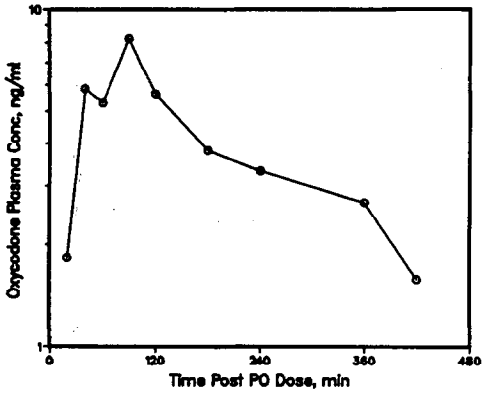

Fig. 3. Plasma concentration *versus* time profile of oxycodone in a healthy volunteer following a single 5-mg oral dose of oxycodone hydrochloride.

Human pharmacokinetic study

The plasma levels of oxycodone in a healthy volunteer after a single oral dose of 5 mg Percocet (oxycodone hydrochloride) are illustrated in Fig. 3. A maximum oxycodone plasma concentration of 8.2 ng/ml was attained at 90 min. The terminal elimination half-life of oxycodone was about 3 h. Plasma oxycodone concentrations (> 1.8 ng/ml) could be detected 7 h after dosing. These results indicate that the assay is sensitive, specific and can be automated. The assay technique is currently being used for bioequivalency studies of different oxycodone formulations in humans.

REFERENCES

1 W. T. Beaver, S. L. Wallenstein, A. Rogers and R. Houde, *J. Pharmacol. Ther.*, 207 (1987) 92.
2 E. Kalso, A. Vainio, M. J. Mattila, P. H. Rosenberg and T. Seppala, *Pharmacol. Toxicol.*, 67 (1990) 322.
3 M. T. Smith, J. A. Watt. G. P. Matt and T. Cramond, *Ther. Drug Monit.*, 13 (1991) 126.
4 N. L. Renzi, Jr. and J. N. Tam, *J. Pharm. Sci.*, 68 (1979) 43.
5 S. H. Weinstein and J. C. Gaylord, *J. Pharm. Sci.*, 68 (1979) 527.
6 J. J. Schneider, E. J. Triggs and D. W. A. Bourne, *J. Chromatogr.*, 308 (1984) 359.
7 U. Saarialho-Kere, M. J. Matilla and T. Seppala, *Pharmacol. Toxicol.*, 65 (1989) 252.