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Note

Determination of oxycodone in human plasma by high-performance liquid chromatography with electrochemical detection

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Oxycodone (14-hydroxydihydrocodeinone) has similar pharmacological properties to morphine and is often used in the treatment of severe pain. Existing methods for the determination of oxycodone in plasma include gas chromatography (GC) with electron-capture [1] or nitrogen-specific detection [2]. While nitrogen-specific detection eliminates the derivatisation step required in electron-capture detection, 5 ml of plasma are needed to detect 2 ng/ml oxycodone. Using the high-performance liquid chromatographic (HPLC) method described in this report, no derivatisation is required and 2 ng/ml oxycodone can be detected using only 2 ml of plasma.

EXPERIMENTAL

Reagents

Stock solutions of oxycodone (1 μg/ml) (Endo Labs., Gordon, Australia) and the internal standard methadone (2 μg/ml) (NBSL, Canberra, Australia) were prepared monthly in methanol and stored at 4°C. Methanol and acetonitrile

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were HPLC grade (Waters Assoc., Brisbane, Australia). Potassium dihydrogen orthophosphate (used in the mobile phase), sodium hydrogen carbonate and sodium carbonate (both used in the carbonate buffer) were AR grade (Ajax Chemicals, Sydney, Australia).

Glassware

Tubes used in the extraction procedure were previously washed with chromium trioxide (Chromerge, Manostate, NY, U.S.A.) in sulphuric acid AR grade (Ajax Chemicals), rinsed thoroughly with distilled water, dried, silanised with 1% hexamethyldisilazine (Ajax Chemicals) in diethyl ether, rewashed in distilled water and dried.

Chromatographic apparatus and conditions

Reversed-phase HPLC was performed using a Model M45 solvent delivery pump (Waters Assoc., Milford, MA, U.S.A.), a Rheodyne Model 7125 injection valve (Rheodyne, Cotati, CA, U.S.A.) equipped with a 40-μl loop, and an RP-8 10-μm column (Brownlee Labs., Santa Clara, CA, U.S.A.). An Omniscribe recorder (Houston Instruments, Austin, TX, U.S.A.) was used in conjunction with an amperometric detector system (Bioanalytical Systems, West Lafayette, IN, U.S.A.). The electrochemical cell contained a glassy carbon working electrode and an Ag/AgCl reference electrode. The working electrode was maintained at an applied voltage of 1.20 V. The chromatography was performed at ambient temperature with a mobile phase of 0.01 *M* potassium dihydrogen orthophosphate—methanol—acetonitrile (20:30:50) at a flow-rate of 1 ml/min. The recorder was set on 10 mV with a chart-speed of 2.5 mm/min.

Extraction method

To 1 ml (or 2 ml) of plasma were added 50 μl of methadone stock solution (internal standard), 500 μl carbonate buffer (pH 9.6) and 6 ml butyl chloride (Burdick & Jackson Labs., Muskegon, MI, U.S.A.). After the mixture was shaken for 15 min on a mechanical shaker (100 rpm), the organic layer was separated by centrifugation (5 min, 2000 *g*) and transferred to a tube containing 3 ml of 0.2 *M* hydrochloric acid. This mixture was shaken for 15 min, centrifuged and the aqueous layer transferred to a tube containing three drops of 60% sodium hydroxide and 6 ml butyl chloride. After shaking for a further 15 min and centrifuging, the organic layer was transferred to a clean tube and the solvent evaporated to dryness at 50° C under a stream of nitrogen. The residue was reconstituted in the HPLC mobile phase (60 μl) and 40 μl were injected into the HPLC system.

Determination of oxycodone in unknown samples

Oxycodone standards (2—200 ng/ml) were prepared by spiking 1-ml or 2-ml aliquots of pooled drug-free plasma with appropriate volumes of oxycodone stock solution. The plasma standards were then extracted and the peak height ratios of oxycodone/methadone (O/M) obtained from the chromatograms were plotted against original oxycodone concentrations. Unknown plasma samples were spiked with the same amount of internal standard and assayed. The oxycodone concentrations of unknown samples were determined from the calibration curve using their peak height ratios (O/M).

RESULTS AND DISCUSSION

Typical chromatograms are shown in Fig. 1. Retention times for oxycodone and methadone are 6.4 minutes and 16 min, respectively. The glassy carbon electrode produced a linear response in the range of 2—200 ng/ml oxycodone in plasma when operated at an applied voltage of 1.2 V. The intra-assay coefficients of variation of the method (C.V.) were 4.1% and 3.6% at oxycodone concentrations of 5 ng/ml (n = 7) and 50 ng/ml, (n = 9), respectively.

The extraction efficiency was 74 ± 3.28% for oxycodone and 80.2 ± 7.15% for methadone (n = 9 for each case). Drugs with similar pharmacological properties were injected and their retention times noted. Three commonly used drugs, fentanyl, phenoperidine and meperidine, had retention times of 6.0, 6.0 and 6.8 min, respectively. To ensure these drugs did not interfere with this assay blank plasma samples were spiked with 100 ng of fentanyl, phenoperidine or meperidine, 100 ng oxycodone and methadone. After extraction and analysis, no interference with the chromatography and quantitation of oxycodone was observed.

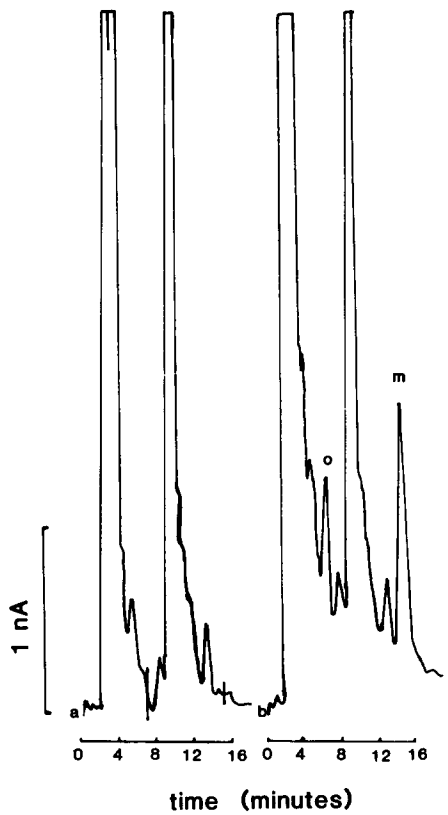

Fig. 1. Chromatograms of 1 ml human plasma taken (a) just prior to administration of oxycodone and (b) 8 h after oral administration of 25 mg oxycodone. Estimated plasma oxycodone concentration is 26 ng/ml. Peaks: o = oxycodone; m = methadone.

Previous methods for the determination of oxycodone in plasma have all used GC. GC with electron-capture detection requires extraction and formation of a volatile oxycodone derivative prior to measurement. In order to determine the pharmacokinetics of oxycodone in human plasma after administration of a single therapeutic dose to normal subjects, repeated blood sampling is required. Using GC with nitrogen-specific detection, 5-ml plasma samples are required at each sampling time. HPLC with electrochemical detection requires only 2-ml plasma samples. Because the total volume of blood withdrawn from a subject is greatly reduced, this method could also be used to study the pharmacokinetics of oxycodone in patients where it is difficult to take very large volumes of blood.

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