Journal of Chromatography, 413 (1987) 309—314
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 3375

Note

Electrochemical determination of oxymorphone in rat plasma by ion-pair reversed-phase high-performance liquid chromatography

G. LAM*, R.M. WILLIAMS and C.C. WHITNEY

Drug Metabolism Section, Stine-Haskell Laboratory, DuPont Pharmaceuticals, P.O. Box 30, Elkton Road, Newark, DE 19714 (U.S.A.)

(First received April 3rd, 1986; revised manuscript received August 4th 1986)

Oxymorphone is a potent narcotic analgesic widely used parenterally for pain relief. Baselt and Stewart [1] developed a gas—liquid chromatographic method with electron-capture detection that is applicable to urine samples where the concentrations are relatively high. Cone et al. [2] described a gas chromatographic—mass spectrometric method to measure the urinary oxymorphone and its metabolites. A lengthy derivatization procedure is required to process the extracts. To date there is no reported assay for oxymorphone in plasma.

The use of electrochemical detection with high-performance liquid chromatography (HPLC) has recently emerged as a preferential method in the analysis of opiate agonists and antagonists. The agonists, morphine [3] and nalbuphine [4—6], and the antagonists, naloxone [6—9] and naltrexone [6, 9], have been successfully analyzed by specific HPLC methods with electrochemical detection. These methods take advantage of the oxidizable phenolic group in the morphinan moiety and yield sensitive quantitations in the ng/ml range. Peterson et al. [10] examined the amperometric responses of various narcotic alkaloids and demonstrated that oxymorphone can also be oxidized.

The present study describes an ion-pair HPLC assay of oxymorphone in rat plasma using electrochemical detection. The method is both sensitive and selective and was applied to rat pharmacokinetic studies.

EXPERIMENTAL

Chemicals and solutions

Oxymorphone hydrochloride and the internal standard, hydromorphone hydrochloride, were obtained from DuPont Pharmaceuticals (Wilmington,

DE, U.S.A.). Analytical-grade chemicals were used to prepare 1 *M* sodium bicarbonate buffer, 0.03 *M* potassium monophosphate, 0.017 *M* phosphoric acid, and 0.3% sodium octane sulfonate.

Diethyl ether, methanol, and acetonitrile were HPLC grade (Burdick & Jackson Labs., Muskegon, MI, U.S.A.) and the water was distilled. Hydromorphone hydrochloride solution (400 ng/ml) was prepared in methanol.

Instrumentation

A DuPont 870 pump (DuPont Instruments, Wilmington, DE, U.S.A.) and a WISP® 710B autosampler (Waters Assoc., Milford, MA, U.S.A.) were used for the analysis. The analytical column was a Regis Hi-Chrom reversible octyl 5-μm (25 × 4.6 mm) column (Regis, Morton Grove, IL, U.S.A.). The mobile phase was 0.03 *M* potassium monophosphate (pH 4)—acetonitrile—0.3% sodium octane sulfonate (25:5:3, v/v/v). The flow-rate was 1.5 ml/min and the column pressure was 170 bar. The eluent was monitored by an electrochemical detector, BAS LC4B, with a glassy-carbon working electrode (BAS, West Lafayette, IN, U.S.A.). The operating oxidation potential was 0.9 V (with reference to Ag/AgCl) and the range was 10 nA. An HP3392A integrator (Hewlett-Packard, Avondale, PA, U.S.A.) was used to record chromatograms and measure peak heights.

Standard solutions and plasma standards

A 1 mg/ml primary stock solution of oxymorphone hydrochloride was prepared by dissolving 10 mg of oxymorphone hydrochloride in methanol in a 10-ml volumetric flask. A series of oxymorphone hydrochloride working standard solutions of concentrations 5, 10, 20, 40, 50, 125, 250, 500, and 1000 ng/ml were prepared by diluting the primary stock solution in methanol accordingly.

Aliquots of 200 μl of the working standard solutions were transferred into 125 × 16 mm borosilicate culture tubes and dried under a gentle stream of air. Heparinized rat plasma (1 ml) was added to each tube and yielded the corresponding oxymorphone plasma standards (free base) of concentrations 0.89, 1.78, 3.57, 7.14, 8.92, 22.3, 44.6, 89.2, and 178.4 ng/ml.

Assay procedure

To 1 ml of plasma sample or standard in a 125 × 20 mm borosilicate culture tube with PTFE-lined screw cap, were added 1 ml of 1 *M* sodium bicarbonate, 200 μl of internal standard (hydromorphone hydrochloride, 400 ng/ml), and 15 ml of diethyl ether. The mixture was rotated for 20 min and then centrifuged (800 *g*) for 15 min. The organic phase was transferred to a 40-ml centrifuge tube containing 200 μl of 0.017 *M* phosphoric acid. The mixture was vigorously mixed for 15 s and then centrifuged for 5 min. The organic phase was discarded and the aqueous phase was dried under a gentle stream of air at 45°C. The residue was reconstituted in 200 μl of 0.017 *M* phosphoric acid, and 100 μl were injected onto the HPLC column.

Calculations

Two unweighted linear regressions were used to determine slopes, intercepts,

and correlation coefficients. One was used to evaluate standard concentrations ranging from 0 to 8.92 ng/ml and the second regression was used to evaluate standard concentrations ranging from 8.92 to 178.4 ng/ml. The peak-height ratios of oxymorphone to hydromorphone were used for all calculations. The concentrations of samples were calculated from the appropriate standard curve.

Animal studies

Three male Sprague—Dawley rats, each weighing about 350 g, were used in the study. The right jugular vein of each rat was cannulated using polyethylene tubing and the cannula was extended to the back of the animal. A bolus injection of oxymorphone hydrochloride (1 mg/kg) was administered through the cannula which was then rinsed with normal saline. Blood samples were taken from the cannula at 2, 5, 15, 30, 60, 120, 180, and 240 min after injection and placed into heparinized tubes. Heparinized blood from a donor rat was transfused into each rat for the blood volume replacement.

RESULTS AND DISCUSSION

Chromatography

Typical chromatograms obtained from a rat plasma blank, plasma spiked with oxymorphone, and a plasma sample after intravenous injection of oxymorphone hydrochloride are presented in Fig. 1. Retention times of oxymorphone and hydromorphone were 7.0 and 8.9 min, respectively. A small peak eluting after hydromorphone was due to an unknown contaminant in the hydromorphone sample. No interfering peak was observed in the chromatogram of the blank plasma. The oxymorphone peak was found in most plasma samples after the intravenous dose.

The pH of the potassium phosphate buffer in the mobile phase was crucial to the peak symmetry. Front tailing often resulted from low buffer pH and back tailing became evident when the pH exceeded 4. The retention volumes of oxymorphone and hydromorphone can be changed by adjusting the strength

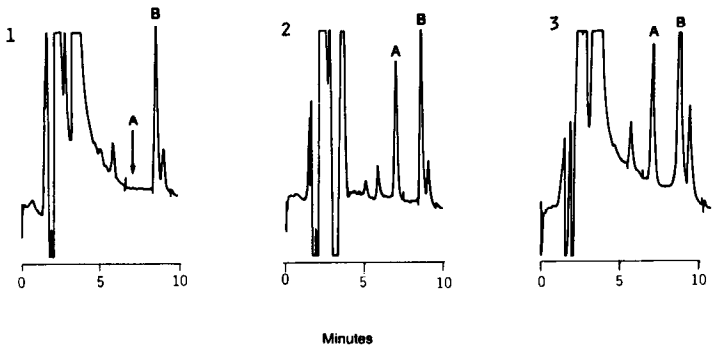

Fig. 1. Chromatograms of (1) rat plasma blank, (2) 8.92 ng/ml oxymorphone and internal standard in rat plasma, and (3) 30-min plasma sample after intravenous dose. Peaks: A = oxymorphone; B = hydromorphone.

of the components of the mobile phase without losing peak shape. The retention volumes can be increased by either decreasing the ionic strength of the buffer and the amount of acetonitrile or increasing the concentration of the ion-pairing reagent.

Throughout the course of the study, the cell in the electrochemical detector lost sensitivity abruptly after a few days of continuous use. Polishing the working electrode, however, returned the cell to its original sensitivity. Exactly what kind of surface reaction was taking place on the working electrode is not known. To avoid this problem we polished the cell daily prior to the analyses of standards and samples.

In the extraction of the plasma sample, we attempted to inject the acid phase directly onto the column after the back-extraction step. This resulted in a huge interfering peak on the chromatogram and rendered the quantitation impossible. By evaporating the acid phase to dryness and then reconstituting the residue for injection, clean chromatograms were obtained.

Linearity

Straight lines were obtained by plotting the peak-height ratio versus the oxymorphone concentration (ng/ml) in plasma for both low and high standard curves. The low standard curve covered concentrations from 0 to 8.9 ng/ml and the high standard curve from 8.9 to 178.4 ng/ml. The correlation coefficients generally exceeded 0.9990.

Precision and accuracy

The precision and accuracy of the method were determined from blank plasma spiked with four different oxymorphone concentrations. These were analyzed in replicate on one day and individually on different days. The concentrations ranged from 1.338 to 133.8 ng/ml. The results are shown in Tables I and II. The within-day precision ranged from 2.4 to 8.5% and the

TABLE I

PRECISION RESULTS OF THE OXYMORPHONE ASSAY

Concentration spiked (ng/ml)	Mean concentration found (ng/ml)	n*	Coefficient of variation (%)
Between-day			
1.34	1.33	5	8.5
6.69	6.34	6	2.6
16.73	16.57	6	5.1
133.80	134.02	6	2.4
Within-day			
1.34	1.30	6	12.2
6.69	6.77	6	2.3
16.73	16.64	6	6.6
133.80	132.00	6	4.0

* n represents the number of days for the between-day results and the number of samples analyzed on one day for the within-day results.

TABLE II

ACCURACY RESULTS OF THE OXYMORPHONE ASSAY

Concentration spiked (ng/ml)	Mean concentration found* (ng/ml)	Mean deviation** (%)
1.34	1.33	9.84
6.69	6.34	1.77
16.73	16.57	3.31
133.80	134.02	3.30

* Between-day precision results were used for accuracy.
** Mean of the absolute percentage deviation from the spiked.

between-day precision ranged from 4.0 to 12.2%. The accuracy of the method was determined from between-day samples. The mean deviation of the found and the spiked concentration ranged from 1.77 to 9.84%.

Rat pharmacokinetic study

The plasma levels of oxymorphone in three rats after intravenous dosing are illustrated in Fig. 2. The plasma concentrations at 2 min after dosing exceeded 1 μg/ml. The oxymorphone levels declined rapidly and reached the terminal log-linear phase at about 60 min. These results indicated the method is suitable for rat pharmacokinetic studies.

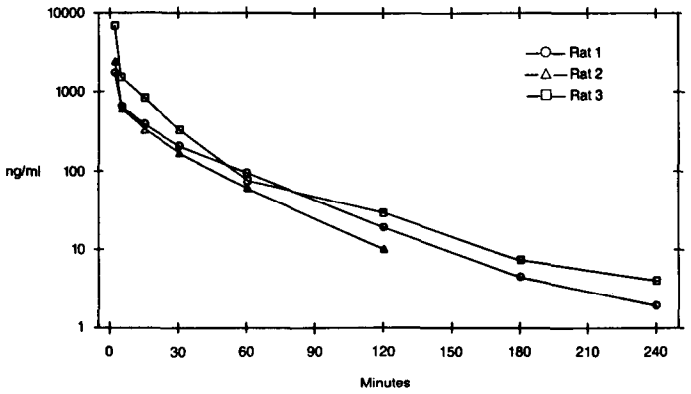

Fig. 2. Plasma concentration versus time profile of oxymorphone in three rats following a single 1 mg/kg intravenous dose of oxymorphone hydrochloride.

ACKNOWLEDGEMENTS

The authors wish to thank Foster Brown and William Flanagan for their assistance in the animal studies.

REFERENCES

1 R.C. Baselt and C.B. Stewart, J. Anal. Toxicol., 2 (1978) 107.

2 E.J. Cone, W.D. Darwin, W.F. Buchwald and C.W. Gorodetzky, Drug Metab. Dispos., 11 (1983) 446.
3 M.W. White, J. Chromatogr., 178 (1979) 229.
4 C.L. Lake, C.A. DiFazio, E.N. Duckworth, J.C. Moscicki, J.S. Engle and C.G. Durbin, J. Chromatogr., 233 (1982) 410.
5 M. Lo, G.P. Juergens and C.C. Whitney, Jr., Res. Commun. Chem. Pathol. Pharmacol., 43 (1984) 159.
6 M. Lo and C.C. Whitney, APhA Acad. Pharm. Sci., 35th Natl. Meet. Abstr., 13 (1983) 190.
7 R.B. Raffa, J.J. O'Neill and R.J. Tallarida, J. Chromatogr., 238 (1982) 515.
8 F.S. Tepperman, M. Hirst and P. Smith, Life Sci., 33 (1983) 1091.
9 H. Derendorf, A.E.A. El-Koussi and E.R. Garrett, J. Pharm. Sci., 73 (1984) 621.
10 R.G. Peterson, B.H. Rumack, J.B. Sullivan, Jr. and A. Makowski, J. Chromatogr., 188 (1980) 420.