ELSEVIER

Journal of Chromatography B, 712 (1998) 169–175

JOURNAL OF CHROMATOGRAPHY B

Solid-phase extraction method with high-performance liquid chromatography and electrochemical detection for the quantitative analysis of oxycodone in human plasma

Andrew W.E. Wright[a], Julie A. Lawrence[b], Michael Iu[a], Tess Cramond[b], Maree T. Smith[a,*]

[a]*School of Pharmacy, The University of Queensland, St. Lucia, Brisbane, 4072 Queensland, Australia*
[b]*Multidisciplinary Pain Centre, Royal Brisbane Hospital, 4029 Queensland, Australia*

Received 13 November 1997; received in revised form 24 February 1998; accepted 17 March 1998

Abstract

A sensitive and reproducible solid-phase extraction (SPE) method for the quantification of oxycodone in human plasma was developed. Varian Certify™ SPE cartridges containing both C_8 and benzoic acid functional groups were the most suitable for the extraction of oxycodone and codeine (internal standard), with consistently high (≥80%) and reproducible recoveries. The elution mobile phase consisted of 1.2 ml of butyl chloride–isopropanol (80:20, v/v) containing 2% ammonia. The quantification limit for oxycodone was 5.3 pmol on-column. Within-day and inter-day coefficients of variation were 1.2% and 6.8% respectively for 284 n*M* oxycodone and 9.5% and 6.2% respectively for 28.4 n*M* oxycodone using 0.5-ml plasma aliquots.

Keywords: Oxycodone

1. Introduction

Oxycodone, an opioid analgesic structurally related to morphine (Fig. 1), is commonly administered to adult patients for the treatment of moderate to severe pain following surgery or for the relief of pain associated with cancer. The analgesic potency of oxycodone has been reported to be approximately 0.5–0.7 that of morphine in adult patients [1,2]. Although this drug has been available for administration to humans since 1917, its clinical pharmacokinetics in adult patients have been defined only in the last 10 years primarily because of the limitations of the detection methods that were available prior to the mid 1980s. Published assays for the quantitation of oxycodone in plasma primarily use gas liquid chromatography with nitrogen–phosphorus detection [3–6] or electron capture detection after the heptafluorobutyryl derivative of oxycodone has been formed. Two high-performance liquid chromatographic (HPLC) methods with electrochemical detection [7,8] have also been reported. Although most of these methods have good sensitivity for the quantitation of oxycodone, they all use large volumes of potentially hazardous organic solvents such as toluene, benzene, butyl chloride and chloroform for the liquid–liquid extraction of oxycodone. Additionally, all of these liquid–liquid extraction methods

*Corresponding author.

0378-4347/98/$19.00
PII: S0378-4347(98)00146-7

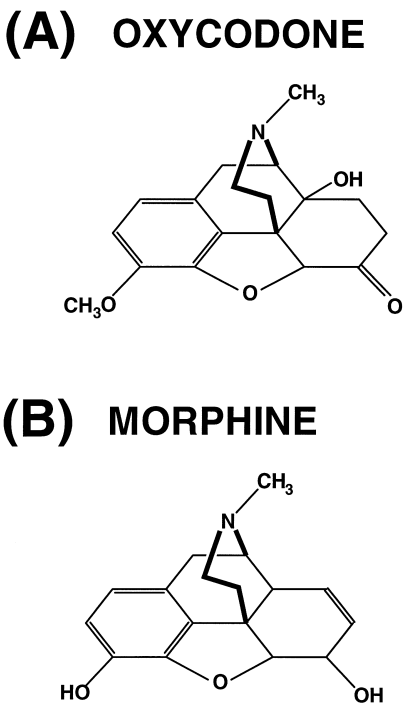

Fig. 1. Chemical structures of (A) oxycodone and (B) morphine.

require at least 1 ml and up to 5 ml of patient plasma. More recently, the suitability of oxycodone for administration to children for postoperative pain relief has been investigated [9]. However, before the clinical pharmacokinetics of this drug can be determined in the paediatric population, a suitable method for the quantitation of the expected low concentrations of this drug in the plasma of children after oral syrup administration must be developed. Clearly collection of large volumes of blood from paediatric patients is not ethically possible. As solid-phase extraction (SPE) is now widely used for biological sample preparation prior to quantitation using HPLC, a SPE approach using only 0.5 ml of plasma was investigated.

2. Experimental

2.1. Chromatography

The HPLC system comprised a Waters 510 solvent delivery system, Waters Wisp 712 automatic injector, Waters Model 460 electrochemical detector (Waters Chromatography, Milford, MA, USA) and a Shimadzu CR4A Chromatopac module (Shimadzu Corporation, Kyoto, Japan). The voltage of the working electrode of the electrochemical detector was set between 1.10 and 1.25 V depending on the age of the KCl in the reference electrode. Chromatographic separations were achieved using a Waters C_{18} Novapak™ cartridge installed in a Waters RCM 10 cm×8 mm radial compression unit with an on-line Guard-Pak™ C_{18} μ-Bondapak guard column, at ambient (23°C) temperature. Peak heights were recorded using the Shimadzu CR4A data module at a chart speed of 1 mm/min. The mobile phase consisted of methanol–acetonitrile–0.0133 *M* phosphate buffer, pH 7.5 (23:2:75, v/v) in which 40 mg/l of cetyltrimethylammonium bromide (cetrimide) was dissolved. The mobile phase was filtered and degassed using 0.45-μm disposable filters in a Millipore solvent filtration apparatus (Millipore-Waters, Milford, MA, USA). Retention times of oxycodone and codeine were 9.6 and 14.7 min, respectively (Fig. 2).

C_2, C_8, C_{18}, CN, anionic and cationic SPE cartridges were purchased from Waters (Sydney, Australia). C_2 and Certify SPE cartridges were obtained from Varian (Sydney, Australia).

2.2. Reagents and standards

Oxycodone hydrochloride was a generous gift from Boots Healthcare (Australia). Codeine was purchased from Sigma (Poole, Dorset, UK). HPLC-grade methanol, acetonitrile and butyl chloride were purchased from Mallinckrodt (Sydney, Australia). All other chemicals were purchased commercially and were of analytical reagent grade. The water used was purified by reverse osmosis followed by removal of soluble organic components and deionisation (≥18 MΩ). Drug-free human plasma was obtained from the Red Cross blood bank (Brisbane, Australia).

Stock aqueous solutions of oxycodone hydrochloride (284 μ*M*) and the internal standard, codeine hydrochloride (298 μ*M*) were prepared in 100-ml volumetric flasks. Seven working dilutions of oxycodone hydrochloride (0.142, 0.284, 0.568, 1.136, 2.272, 4.54 and 5.68 μ*M*) were also prepared in

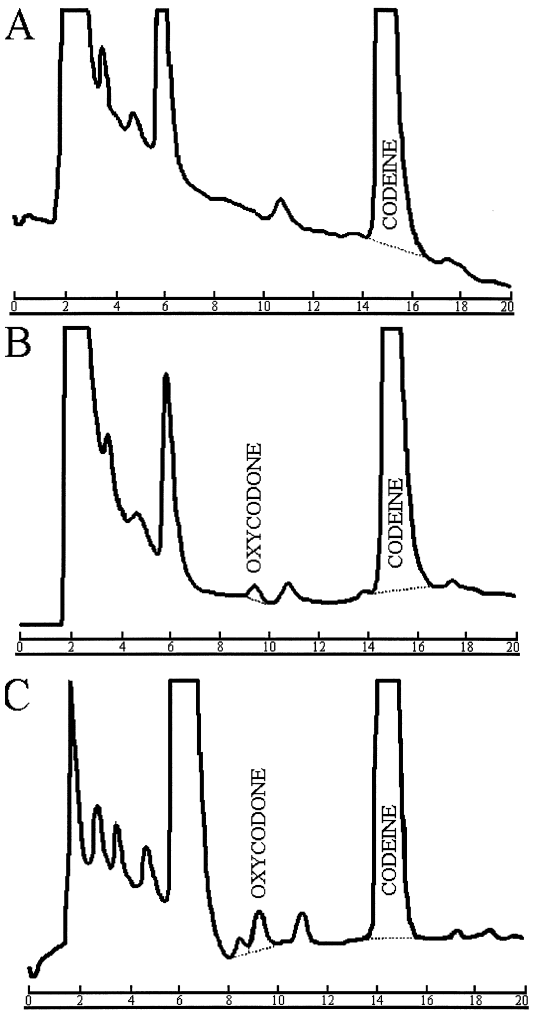

Fig. 2. (A) Drug-free human plasma (0.5 ml) to which codeine has been added in a concentration of 595.6 n*M*. Full scale deflection= 4.4 mV. (B) Drug-free human plasma (0.5 ml) to which oxycodone and codeine has been added in concentrations of 14.2 n*M* and 595.6 n*M*, respectively. Full scale deflection=4.4 mV. (C) Paediatric human plasma (0.5 ml) containing oxycodone and codeine in concentrations of 42.9 n*M* and 595.6 n*M* respectively. Full scale deflection=4.4 mV.

100-ml volumetric flasks using deionised water. Calibration standards were prepared by adding 50-μl aliquots of the working dilutions of oxycodone hydrochloride to polypropylene assay tubes (10 ml) and adding 500 μl of drug free plasma. Aliquots (50 μl) of the internal standard working dilution (5.96 μ*M* codeine hydrochloride) were added to all assay tubes. Human plasma containing oxycodone hydrochloride (28.4, 56.8 and 284 n*M*) were prepared as low, medium and high concentration quality control samples, respectively.

2.3. Ethical approval

Ethical approval for the study of the administration of oxycodone syrup to children for the relief of postoperative pain was obtained from the Ethics Committee, Royal Children's Hospital and the Human Experimentation Ethical Review Committee of The University of Queensland.

2.4. Sample handling and extraction procedure

Blood samples were collected via a cannula inserted into a forearm vein immediately predosing and at the following postdosing times: 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 8, 12 and 24 h. Blood samples were centrifuged, the plasma separated and transferred via polypropylene pipettes to plain polypropylene tubes. Plasma samples were stored at −20°C until the time of analysis. Plasma samples were extracted using Certify (Varian) SPE cartridges housed in a Vac-Elut vacuum system (Analytichem International, Harbor City, CA, USA). The Vac-Elut system produced a reproducible vacuum (1–10 in. Hg) for up to 10 SPE cartridges simultaneously. Extraction cartridges were conditioned with 2 ml of methanol followed by 2 ml of 0.1 *M* phosphate buffer (pH 8.0).

Plasma samples were thawed just prior to assay. Polypropylene tubes were used at all stages of the assay to prevent potential adsorptive losses of the weak bases, oxycodone and codeine onto glass surfaces. Aliquots (0.5 ml) of plasma (standards or samples) were added to 10-ml polypropylene centrifuge tubes and 50-μl aliquots of the internal standard (5.96 μ*M* codeine hydrochloride) were added to each tube. After addition of 1 ml of 0.1 *M* phosphate buffer (pH 8.0), samples were vortexed for 10 s and loaded onto the conditioned SPE cartridges under a gentle vacuum (<1 in. Hg). Extraction cartridges were washed with 5 ml of 0.1 *M* phosphate buffer (pH 8.0), then 1 ml of deionised water followed by 2 ml of 0.1 *M* acetate buffer (pH 4.0), then 2 ml of methanol. Extraction cartridges

were then dried under a moderate vacuum (>6 in. Hg) for 2–4 min. A 200-μl wash with the SPE elution phase (butyl chloride–isopropanol (80:20, v/v) containing 2% ammonia) removed endogenous plasma constituents without the loss of oxycodone or codeine from the extraction cartridges. The cartridges were then dried under vacuum as completely as possible (>6 in. Hg for 2–4 min) to ensure all of the 200-μl wash was removed prior to the elution of oxycodone and codeine with a subsequent 1.2-ml aliquot of the elution phase. The eluent, collected in 1.5-ml polypropylene microfuge tubes, was evaporated to dryness under a stream of high purity nitrogen at room temperature (23°C) and the dried residues were reconstituted in 200 μl of HPLC mobile phase, 150 μl of which was injected into the HPLC.

3. Results

Preliminary studies in the development of this assay involved the evaluation of the performance of a range of different SPE cartridges with respect to (i) recovery of oxycodone and codeine, and (ii) 'cleanliness' of the solid-phase plasma extract. These investigations showed that the Varian Certify cartridges containing both C_8 and benzoic acid functional groups were superior to C_2, C_8, C_{18}, cationic and anionic extraction cartridges for overall performance as assessed not only by the retention of oxycodone on the extraction cartridge during the wash steps but also by the subsequent elution of oxycodone in a relatively small volume of elution mobile phase. Anionic, cationic, C_2 and CN cartridges failed to retain oxycodone sufficiently whereas oxycodone became progressively more difficult to elute from C_8 ad C_{18} extraction cartridges.

3.1. Detector potential

We have previously determined that with fresh KCl solution in the reference electrode of the electrochemical detector, the plateau region for oxycodone's voltammogram was in the range 1.15–1.25 V [8]. However, it must be remembered that as the KCl solution in the reference electrode ages, voltammograms become displaced to the left, such that the nominal voltage required to oxidise oxycodone is reduced. To ensure reproducible oxidation of oxycodone in the electrochemical detector on a day-to-day basis, the working voltage of the electrochemical detector was adjusted to ensure that a peak height of 700 μV was obtained for 28.4 pmol of oxycodone hydrochloride injected on-column into the HPLC.

3.2. Column preparation and composition of mobile phase

Consistent with our previous findings [8,10], each new Novapak C_{18} cartridge had to be deactivated prior to its use for quantitative analysis. This involved the addition of a high concentration of cetrimide (1 g/l) to the mobile phase and recycling this fluid continuously through the analytical column and the guard column, at a flow-rate of 2 ml/min, for at least 24 h. This procedure reduced excessive tailing of oxycodone in particular, by effectively coating unendcapped SiOH groups on the stationary phase of the column. After saturation with cetrimide, the column was equilibrated with the mobile phase running to waste for a further 24–36 h. Thereafter, mobile phase containing 40 mg/ml of cetrimide was sufficient to maintain a reproducible effect. An in-line Waters Guard-Pak C_{18} μ-Bondapak guard column was used to maximise the analytical column life. After replacing the guard column cartridge, 20–30 ml of mobile phase containing cetrimide (1 g/l) was passed through the guard column (after wetting with 100% methanol followed by 100% water) to effectively endcap free SiOH groups, before placing the guard column back in-line.

As the number of theoretical plates in an analytical column gradually decreases with continued column use, it was necessary to periodically compensate for this by making minor changes to the mobile phase composition such as decreasing the methanol content (1–2% increments) or increasing the pH (0.1 pH unit increments) or increasing the cetrimide concentration (1 mg/l increments), in order to maintain good resolution between oxycodone and a peak in the chromatogram due to an endogenous component of paediatric plasma (Fig. 2). Due to the relative 'cleanliness' of the solid-phase extracts of human plasma using this method, it was possible to recycle

mobile phase for 48 h without deactivating the surface of the glassy carbon electrode.

3.3. Sample handling

Many weakly basic compounds bind to glass surfaces, particularly in low concentrations such as the concentrations of oxycodone reportedly found in patient plasma [11,12]. As oxycodone binds avidly to glassware (data not shown), polypropylene tubes were used throughout this assay to prevent possible adsorptive losses of the compounds of interest onto glass surfaces. However, when glass volumetric flasks were used to make stock drug solutions, they were pretreated overnight in a saturated aqueous solution of cetrimide and then rinsed thoroughly in deionised water.

3.4. Recovery

The efficiency of the solid-phase extraction of oxycodone and codeine from human plasma using Certify SPE cartridges ($n=10$) was determined by comparing peak heights of each compound eluted from these cartridges with those obtained from nonextracted oxycodone and codeine solutions (in mobile phase) after addition to the dry residues obtained after SPE of drug-free human plasma. Recoveries (mean±S.D.) of oxycodone and codeine were approximately 80% (Table 1). Typical chromatograms illustrating (a) drug-free human plasma (0.5 ml), (b) drug-free human plasma (0.5 ml) containing oxycodone and codeine in concentrations of 14.2 n*M* and 595.6 n*M* respectively and (c) paediatric human plasma (0.5 ml) containing oxycodone and codeine in concentrations of 42.9 n*M* and 595.6 n*M* respectively, are shown in Fig. 2. The specificity of this HPLC assay for the quantification of oxycodone is the same as that reported previously by our laboratory when using liquid–liquid extraction [8].

3.5. Precision, accuracy and detection limit

Aqueous solutions containing oxycodone and codeine were injected sequentially into the HPLC ($n=10$) in volumes of 150 μl. Coefficients of variation (C.V.s) of the peak heights were <6.3% for each compound (data not shown). The lower limit of quantitation (10 times baseline noise) for oxycodone extracted from human plasma (0.5 ml) was 14.2 n*M*. Human plasma samples (0.5 ml, $n=5$–8) containing oxycodone in five concentrations within the range 14.2–568.4 n*M* were assayed on the same day and

Table 1
Recovery, intra-assay precision and inter-assay accuracy for quantitation of oxycodone in human plasma

Compound	pmol on column	Mean (%)	S.D. (%)	C.V. (%)	*n*	Error (%)
Recovery						
Oxycodone	28.4	80.9	6.0	7.4	10	
	142	79.8	3.9	4.8	10	
	284	83.9	2.6	3.1	10	
Codeine	298	79.6	5.8	7.6	10	
Intra-day variation						
[oxycodone] n*M*	14.2	15.26	3.87	25.3	8	7.4
(0.5-ml samples)	28.4	25.70	2.44	9.5	5	−9.5
	56.8	55.54	3.13	5.6	5	−2.2
	284	302.72	3.72	1.2	5	6.6
	568	594.13	30.07	5.1	5	4.6
Inter-day variation						
[oxycodone] n*M*	14.2	14.18	4.66	32.9	8	−0.1
(0.5 ml samples)	28.4	29.08	1.79	6.2	6	2.4
	56.8	56.56	8.78	15.5	6	−0.4
	284	287.43	19.47	6.8	6	1.2

C.V.=coefficient of variation, n=number of replicates, error (%)=percentage error from nominal concentration.

the peak height ratios determined. The respective within-run C.V.s (calculated from the daily standard curve) for these concentrations of oxycodone are shown in Table 1. Specifically, at the lowest concentration evaluated (14.2 n*M*) the within-run C.V. was 25% whereas for the remaining oxycodone concentrations evaluated the C.V.s were <10%. Between-run data obtained from human plasma samples (0.5 ml) from the low, medium and high control samples incorporated into each chromatographic run gave C.V.s that were <16% (Table 1). Additionally, the inter-day C.V. for the LOQ (14.2 n*M*) was determined to be approximately 33% (Table 1).

3.6. Linearity

Linearity of oxycodone standard curves was determined using least-squares regression analysis of peak-height ratios (drug to internal standard) versus drug concentration in plasma. Correlation coefficients of the standard curves were typically ≥0.995 for seven concentrations of oxycodone.

4. Discussion

This SPE method for the quantitation of oxycodone in human plasma has several advantages compared with liquid–liquid extraction of oxycodone from human plasma. Specifically, our SPE method results in high (≈80%) and reproducible extraction of oxycodone and the internal standard codeine. The detection limit of oxycodone in human plasma is 5.3 pmol injected on-column which provides sufficient sensitivity to permit the quantitation of the plasma concentrations of oxycodone achieved in children dosed with oxycodone syrup (0.25 mg/kg) for postoperative pain relief following ear, nose and throat surgery. A representative plasma oxycodone concentration versus time curve after a single oral syrup dose of oxycodone (6.1 mg) to one paediatric patient undergoing tonsillectomy is shown in Fig. 3.

Additionally this SPE of oxycodone from human plasma uses only 1.4 ml of butyl chloride per sample compared with the 10 ml required previously for our liquid–liquid extraction method [8], thereby significantly reducing solvent costs. Furthermore, due to the 'cleanliness' of the solid-phase extracts, the HPLC mobile phase could be recycled for a period of 48 h, making an additional contribution to the reduction in solvent usage and costs.

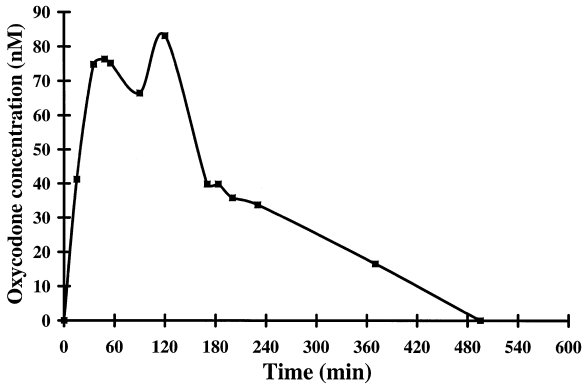

Fig. 3. Representative plasma oxycodone concentration versus time curve obtained in a paediatric patient following oral administration of oxycodone syrup (0.25 mg/kg) in a dose of 6.1 mg, approximately 1 h prior to induction of anaesthesia for tonsillectomy.

In a manner analogous to that reported previously by our laboratory [8], saturation of the Novapak C_{18} cartridge with cetrimide and addition of cetrimide to the mobile phase was essential to reduce the strong H-bonding interactions between oxycodone and unendcapped Si–OH groups in the stationary phase, so that oxycodone could be eluted from the HPLC column with a Gaussian peak shape and a marked reduction (>3-fold) in the organic content of the mobile phase. Retention times of oxycodone and codeine could be selectively increased or decreased relative to the retention times of the small peaks in the chromatogram due to endogenous components of plasma by adjusting the pH in increments of 0.1 pH units. These small changes in pH altered the proportion of each opioid that was ionised (quaternized ring nitrogen), thereby altering retention times.

Codeine was selected as the internal standard, as it is usually not coadministered with oxycodone and it is neither a metabolite nor a degradation product of oxycodone. However, if a patient had also ingested codeine with oxycodone, it would be detected by assaying some plasma samples without the addition of internal standard and by ensuring that the peak

height of the internal standard was within the C.V. of the assay.

Our findings of the superior performance of Certify SPE cartridges for the quantitative analysis of oxycodone from human plasma is consistent with the findings of Huang et al. [13] for the screening of urine samples for opiates. Currently, this assay is being used routinely in our laboratory to quantify concentrations of oxycodone in the plasma of both adults and children following administration of oxycodone for pain relief.

Acknowledgements

Financial support was provided by Pharmaceutical Defence limited, The Australian and New Zealand College of Anaesthetists and The University of Queensland Research Grants Scheme.

References

[1] W.T. Beaver, S.L. Wallenstein, A. Rogers, R.W. Houde, J. Pharmacol. Exp. Ther. 207 (1978) 92.
[2] E. Kalso, R. Poyhia, P. Onnela, K. Linko, I. Tigerstedt, T. Tammnisto, Acta Anaesthesiol. Scand. 35 (1991) 642.
[3] N.L. Renzi Jr., J.N. Tam, J. Pharm. Sci. 68 (1979) 43.
[4] S.H. Weinstein, J.C. Gaylord, J. Pharm. Sci. 68 (1979) 527.
[5] U. Saarialho-Kere, M.J. Mattila, T. Seppälä, Pharmacol. Toxicol. 65 (1989) 252.
[6] R.P. Kapil, P.K. Padovani, P. King, Shang-Ying, G.N. Lam, J. Chromatogr. 577 (1992) 283.
[7] J.J. Schneider, E.J. Triggs, D.W.A. Bourne, J. Chromatogr. 308 (1984) 359.
[8] M.T. Smith, J.A. Watt, G. Mapp, T. Cramond, Ther. Drug. Monitor. 13 (1990) 126.
[9] A.M. Blinco. B. Med. Sci. Thesis, The University of Queensland, 1996.
[10] A.W.E. Wright, J.A. Watt, M. Kennedy, T. Cramond, M.T. Smith, Ther. Drug. Monitor. 16 (1994) 200.
[11] K.P. Leow, M.T. Smith, J.A. Watt, B.E. Williams, T. Cramond, Ther. Drug. Monitor. 14 (1992) 479.
[12] K.P. Leow, M.T. Smith, B.E. Williams, T. Cramond, Clin. Pharmacol. Ther. 52 (1992) 487.
[13] W. Huang, W. Andollo, W.L. Hearn, J. Anal. Toxicol. 16 (1992) 307.