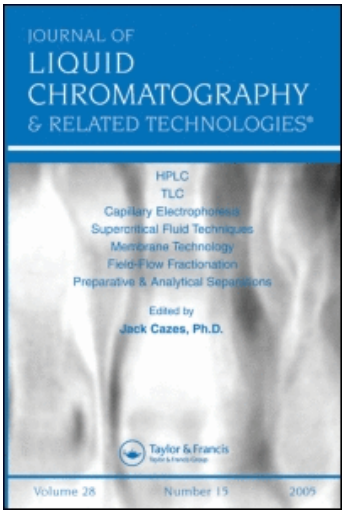

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Determination of Oxycodone and Its Metabolite, Noroxycodone, in Human Plasma by HPLC with Post-column Chemiluminescence Detection Using Electrogenerated Tris(2,2'-Bipyridyl)ruthenium(III)

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Determination of Oxycodone and Its Metabolite, Noroxycodone, in Human Plasma by HPLC with Post-column Chemiluminescence Detection Using Electrogenerated Tris(2,2′-Bipyridyl)ruthenium(III)

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ABSTRACT

A sensitive high performance liquid chromatography (HPLC) method with post-column chemiluminescence detection was developed for the simultaneous determination of oxycodone (OXC) and its metabolite, noroxycodone (NOC), in human plasma. Sample preparation for human plasma was performed by means of solid extraction with Sep-Pak Vac C18 (100 mg/mL). Separation was performed by reversed-phase HPLC

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using a 5-μm Develosil ODS-HG-5 column (150 mm × 4.6 mm) and a mobile phase consisting of a mixture of 0.1 mol/L phosphate buffer (pH 2.6) containing 5 mmol/L sodium dodecylsulfate and methanol (53 : 47, v/v). Quantitation was performed by the measurement of chemiluminescence using electrogenerated tris(2,2′-bipyridyl)ruthenium(III). The lower and upper limits of quantitation for OXC and NOC were 0.5–250 ng/mL and 1–500 ng/mL, respectively. The calibration curve for this assay showed good linearity for the concentration range of 0.5–50 ng/mL for OXC and 1–100 ng/mL for NOC. Recoveries of the studied compounds were excellent at the individual assay ranges (102.9–108.7%), and validation of the assay gave results that were satisfactory in terms of within-run or between-run precision (1.9–11.9% as RSD) and accuracy (−13.5% to +9.2% as bias). Stability studies showed that OXC and NOC were stable in assay solutions for at least 6 days at 4°C storage in the autosampler. OXC and NOC were also stable in human plasma for at least 48 hr at room temperature, for at least 8 months at −20°C and after five repeated freeze–thaw cycles. Furthermore, OXC and NOC were stable in human whole blood for up to 5 days at 4°C and for up to 5 hr at room temperature. This system was applied to the clinical study of cancer patients after single oral administration doses of controlled-released tablets of OXC hydrochloride. Consequently, this method should serve as a useful tool for pharmacokinetic studies of OXC and NOC.

Key Words: Oxycodone; Noroxycodone; Post-column chemiluminescence; Human plasma.

INTRODUCTION

Oxycodone (OXC) is a synthetic opioid agonist that has been available for clinical use since 1917 and has been used widely in a variety of formulations as an antitussive and analgesic. It has been used as an alternative to morphine, which is a representative strong opioid for cancer-related pain, for several reasons. The oral bioavailability of OXC in humans is better than that of morphine, and its elimination half-life is longer.[1–4] Therefore, the analgesic effect of OXC can be observed at a lower oral administration dosage than morphine.[5] Also, unchanged OXC contributes mainly to the analgesic effect, making it easier to control pain than with morphine, which works via its major active metabolite, morphine-6-glucuronide. OXC has been confirmed to be metabolized in humans by *O*-demethylation, *N*-demethylation, *N*-oxidation, 6-keto reduction and glucuronidation. Although the *N*-demethylated metabolite, noroxycodone (NOC), is mainly metabolized in human urine and plasma, NOC is not thought to contribute to the analgesic effect. In

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contrast to NOC, the *O*-demethylated metabolite, oxymorphone, has a significant analgesic effect, but its concentration is negligible.[6–8]

Several chromatographic methods have been proposed for the quantitation of OXC in human plasma including high performance liquid chromatography (HPLC),[9–11] gas chromatography,[12,13] and liquid chromatography/tandem mass spectrometry.[14] None of them has been satisfactory from the pharmacokinetic viewpoint with respect to sensitivity, sample size or simplicity of pretreatment.

We have focused on chemiluminescence detection using electrogenerated tris(2,2′-bipyridyl)ruthenium(III), which can selectively detect the tertiary amine group.[15,16] We have tried to use it for an analytical method for the determination of OXC and NOC by an HPLC system with post-column chemiluminescence detection.[17] However, the system had some drawbacks. The peak frontings of OXC and NOC were marked above pH 2, although the optimum pH for detection was pH 6. To optimize the condition, we adopted a three-pump system, consisting of a pH 1.9 mobile phase, $Ru(bpy)_3^{2+}$ solution and a pH-conditioning solution (pH 5.9). The pH had to be precisely adjusted, but it was difficult to control such a complex system. Therefore, the aim of this study was to solve these problems, simplify the HPLC system, and develop a useful routine analysis system for human plasma samples.

This report describes an improved HPLC method with post-column electrogenerated chemiluminescence detection for the quantitative determination of OXC and its metabolite, NOC, in human plasma, together with the validation results. The suitability of these compounds for use in clinical studies is also discussed.

EXPERIMENTAL

Materials and Reagents

The chemical structures of the compounds studied are shown in Fig. 1. OXC hydrochloride was obtained from Sankyo (Tokyo, Japan), and NOC was synthesized at Shionogi (Osaka, Japan). Tris(2,2′-bipyridyl)ruthenium(II) chloride hexahydrate [$Ru(bpy)_3Cl_2$] was obtained from Fluka Chemie (Buchs, Switzerland). Biochemically prepared phosphoric acid and biochemically prepared sodium dodecylsulfate (SDS) were purchased from Wako Pure Chemical Industries (Osaka, Japan). HPLC-grade methanol was purchased from Kanto Chemical (Tokyo, Japan). Guaranteed reagent grade sodium dihydrogenphosphatedihydrate and HPLC-grade distilled water were obtained from Nacalai Tesque (Kyoto, Japan). Guaranteed reagent grade sulfuric acid was obtained from Yoneyama (Osaka, Japan).

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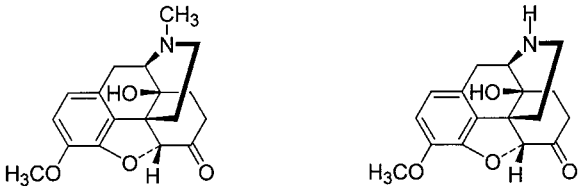

Figure 1. Chemical structures of OXC and NOC.

Instruments and Operational Conditions

The scheme of the HPLC system is shown in Fig. 2. A L-6000 pump (Hitachi, Ibaragi, Japan) was used to deliver the mobile phase of 0.1 mol/L phosphate buffer containing 5 mmol/L SDS (pH 2.6) and methanol (53 : 47, v/v) at a flow rate of 1.0 mL/min. Samples were injected via a WISP 710B autosampler (Waters, MA) at 4°C. Develosil ODS HG-5, 150 mm × 4.6 mm i.d., 5-μm particle size (Nomura Chemical, Aichi, Japan) was used as the analytical column with a precolumn filter (Shiseido-Irica Technology, Kyoto, Japan). The column temperature was maintained at 50°C using a U-620 column heater (Sugai, Tokyo, Japan). The post column reaction reagent solution was prepared by dissolving 0.75 mmol/L $Ru(bpy)_3Cl_2$ in 0.5 mmol/L sulfuric acid solution. The solution was degassed with a DGU-10B He degasser (Shimadzu, Kyoto, Japan) and delivered by a L-6200 pump (Hitachi, Ibaragi, Japan) at a flow rate of 0.3 mL/min through a Model 5020 carbon graphite

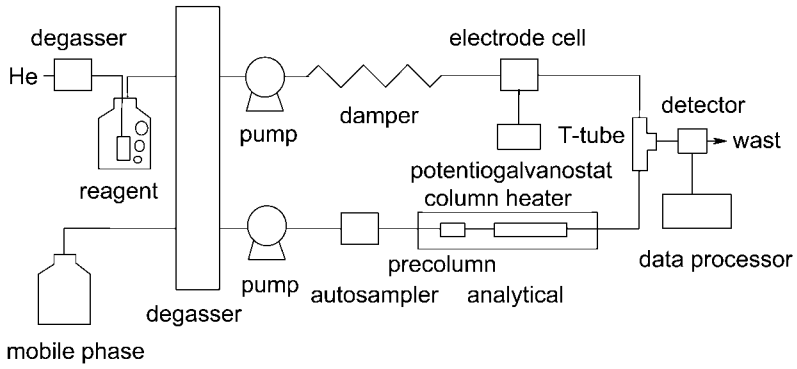

Figure 2. HPLC system with chemiluminescence detection using electrogenerated *tris*(2,2′-bipyridyl) ruthenium(III).

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electrode cell (ESA, Bedford). The reagent was mixed with the mobile phase via a T-tube, and then the mixture was introduced to a chemiluminescence detector CLD-10A (Shimadzu, Kyoto, Japan) controlled at the photomultiplier voltage of 0.5 kV. The electrode cell was operated at 150 μA using a Model NPGS-2501 potentiogalvanostat (Nikkokeisoku, Kanagawa, Japan). A Shodex DP-1 damper (Showa Denko, Tokyo, Japan) was set to eliminate the pulsation. Millennium software (Waters, MA) was used as the data processor. A DGU-3A (Shimadzu, Kyoto, Japan) was used as the degasser for all solutions.

Preparation of Standard Solutions

Standard Solutions for Calibration

One milligram each of OXC and NOC as a free base was accurately weighed and dissolved in 10 mL of water to prepare stock solutions (0.1 mg/mL). Standard solutions for calibration of seven concentrations were prepared by dilution of the stock solutions with water (1.25, 2.5, 5, 12.5, 25, 50, 125 ng/mL of OXC and 2.5, 5, 10, 25, 50, 100, 250 ng/mL of NOC). A 100-μL aliquot of each solution was used to prepare a calibration curve.

Standard Solutions for the Recovery Test

A 0.5-mL portion of each standard solution for calibration was diluted to 5 mL with water. A 100-μL aliquot was used to obtain a calibration curve for the recovery test.

Assay Procedure

A Sep-Pak Vac C18 cartridge (packing weight: 100 mg/1 mL, Waters, MA) was placed on a Visiprep-DL solid phase extraction vacuum manifold (Supelco, PA). A solution consisting of 250 μL of sample plasma, 100 μL of water (or standard solution in the case of calibration samples) and 500 μL of water was applied to each cartridge conditioned previously with 1 mL of methanol, 1 mL of 10 mmol/L phosphate buffer (pH 2.0)/methanol (1 : 1, v/v) and 1 mL of water. This solution was aspirated in vacuo. The cartridge was washed with 5 mL of water and 2 mL of 10 mmol/L phosphate buffer (pH 2). The analytes were then eluted with 1 mL of 10 mmol/L phosphate buffer (pH 2)/methanol (1 : 1, v/v). A 100-μL aliquot of the assay solution was injected into the HPLC system.

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Calibration Curve

A 100-μL portion of each standard solution was added to 250 μL of control plasma, and the mixture was treated in the same manner as described in the assay procedure. The calibration curve was obtained as a function of peak area and the concentration of OXC or NOC through the weighted least squares method with a weighting factor of $1/x^2$ at a concentration ranging from 0.5 to 50 ng/mL for OXC and from 1 to 100 ng/mL for NOC in human plasma.

Assay Validation

Within- and Between-Run Precision and Accuracy

Within-run precision and accuracy were evaluated on the basis of the determined values obtained from analysis of five replicates per concentration level of validation samples spiked with OXC and NOC at levels of 0.5, 5, 50, 250 ng/mL and 1, 10, 100, 500 ng/mL, respectively. The between-run precision and accuracy of the assay were evaluated by analyzing the validation samples over three runs. All 15 results of the three runs were used for the evaluation of between-run precision and accuracy.

Recovery of OXC and NOC from Sample Plasma

The standard solutions for the recovery test (100 μL each) were injected in order to obtain a series of the peak areas of OXC and NOC. The calibration curve for the recovery test was obtained as a function of peak area and the concentration corresponded to the plasma concentration of OXC and NOC through the weighted least-squares method with a weighting factor of $1/x^2$ at a concentration ranging from 0.5 to 50 ng/mL for OXC and from 1 to 100 ng/mL for NOC in human plasma, respectively. Using these calibration curves for the recovery test and the peak areas of OXC and NOC obtained from measurement of the five replicates per concentration level of validation samples in the run for the evaluation of the within-run precision and accuracy, the concentrations of OXC and NOC for the determination of the recovery were calculated. The recoveries were calculated as the relative percentages of the mean calculated concentrations compared to the theoretical concentrations.

Stability in Assay Solutions

Samples for the stability test of the assay solution were prepared with human plasma containing OXC and NOC at levels of 1.25, 40 ng/mL and

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2.5, 80 ng/mL, respectively. These samples were treated according to the assay procedure, then stored at 4°C for 6 days and re-injected.

Stability in Plasma

Samples for the stability test of plasma subjected to freeze–thaw cycles were prepared with OXC and NOC at levels of 1.25, 50, 250 ng/mL and 2.5, 100, 500 ng/mL, respectively. Some samples were assayed prior to freezing. The remaining samples were frozen at −40°C, then thawed at room temperature and assayed after three or five freeze–thaw cycles.

Samples for the stability test of plasma stored at room temperature were prepared with OXC and NOC at levels of 1.25, 50, 250 ng/mL and 2.5, 100, 500 ng/mL, respectively. An aliquot of each concentration was stored at room temperature for up to 48 hr and then assayed.

Samples for the stability test of plasma stored at −20°C were prepared with OXC and NOC at levels of 1, 5, 40 ng/mL and 2, 10, 80 ng/mL, respectively. Aliquots of the samples at each concentration were stored at −20°C for up to 8 months and then assayed.

Stability in Whole Blood

Samples for the stability test in whole blood stored at 4°C and room temperature were prepared with OXC and NOC at levels of 1.25, 50, 250 ng/mL and 2.5, 100, 500 ng/mL, respectively. Aliquots of each concentration were stored at 4°C for 0, 2, 5, 7 days and then assayed.

Aliquots of the samples at each concentration were stored at room temperature for 0, 5, 24, 48 hr and then assayed.

RESULTS AND DISCUSSION

Analytical Conditions

In the previous assay method for dog plasma, OXC and NOC were detected under neutral conditions for the maximum intensity of ECL.[17] However, there are few species with electrogenerated chemiluminescent (ECL) intensity under acidic conditions, and compounds with ECL intensity under acidic condition can be detected more selectively without being affected by interference under the acidic condition. After examination of the effects of pH, we concluded that pH 2.6 was appropriate for the mobile phase. Under these conditions, OXC and NOC can be detected with high sensitivity and no interference peak.

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Broglé et al.[18] reported that gem-diol and hemiketal adducts of OXC were formed as a result of reversible addition of water and methanol to the C-6 ketone in pH 3 mobile phase and caused peak fronting. The interconversion of these species is temperature-dependent. Therefore, we checked the relationships between column temperature and peak shape in our system. As shown in Fig. 3, even if pH of the mobile phase is above 2, at which pH peak fronting of OXC was observed at room temperature, we are able to see a symmetrical peak at above 50°C. Therefore, the column temperature was set at 50°C.

Based on these findings, a simple post-column HPLC system (Fig. 2) for the simultaneous determination of OXC and NOC in human plasma was developed without the need for a pH control buffer solution.

Selectivity

The selectivity of the assay was checked by analyzing eleven independent blank human plasma samples. Figure 4 shows the typical chromatograms of blank plasma, blank plasma spiked with drugs and plasma obtained from a patient. There were no marked peaks at the retention times of OXC and

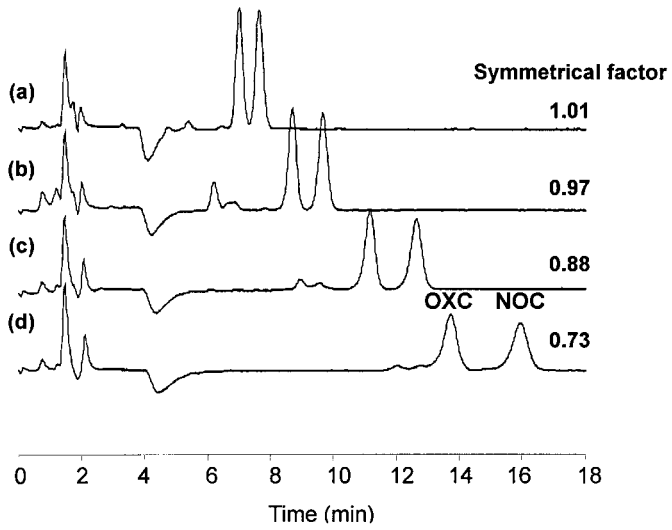

Figure 3. Chromatograms of OXC and NOC at (a) 60°C, (b) 50°C, (c) 40°C, and (d) 30°C under the HPLC conditions described in the Experimental section. Symmetrical factor of OXC at each temperature was calculated with Millennium software.

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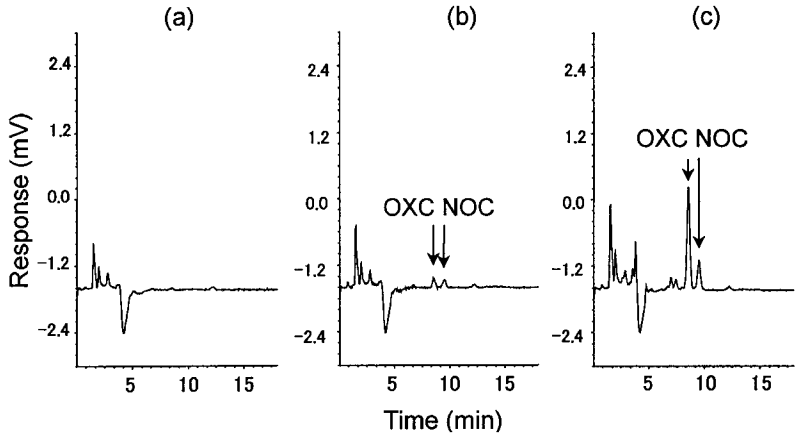

Figure 4. Chromatograms of (a) blank plasma, (b) blank plasma spiked with 0.514 ng/mL OXC and 1.04 ng/mL NOC and (c) plasma obtained from a patient at 5 hr after a single 5-mg oral dose of OxyContine tablet (6.22 ng/mL OXC and 3.84 ng/mL NOC).

NOC. The assay has selectivity for separating OXC and NOC from endogenous plasma components.

Linearity

All standard curves generated in the validation process were linear at the OXC and NOC concentration range of 0.5–50 ng/mL and 1–100 ng/mL, respectively. The bias between the back-calculated concentrations and the theoretical values were within ±6.2%. The coefficient of correlation of each compound was higher than 0.99.

Within- and Between-Run Precision and Accuracy

The precision and accuracy results are listed in Table 1. The within-run precision as RSDs for OXC and NOC in human plasma ranged from 1.9% to 5.8% and from 1.9% to 5.8%, respectively, at each concentration level. The within-run accuracy for OXC and NOC ranged from −2.3% to +6.1% and from −13.5 to +9.2, respectively. The between-run precision as RSD for OXC and NOC in human plasma ranged from 2.4% to 10.6% and from 3.8% to 11.9%, respectively, at each concentration level. The between-run

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Table 1. Method validation results for human plasma.

		Within-run ($n = 5$)				Between-run ($n = 5$)		
Compound	Spiked conc. (ng/mL)	Measured conc. (ng/mL)	Precision (RSD, %)	Accuracy (Bias, %)	Recovery (%)	Measured conc. (ng/mL)	Precision (RSD, %)	Accuracy (bias, %)
OXC	0.527	0.515	5.8	−2.3	106.5	0.492	10.6	−6.6
	5.27	5.48	2.2	+4.0	107.0	5.24	3.8	−0.6
	52.7	53.8	1.9	+2.1	104.4	53.3	2.4	+1.1
	264	280[a]	2.1	+6.1	108.7	267[a]	4.1	+1.1
NOC	1.03	0.891	5.8	−13.5	106.8	0.909	11.9	−11.7
	10.3	10.7	2.8	+3.9	103.9	10.2	3.9	−1.0
	103	108	1.9	+4.9	102.9	104	3.8	+1.0
	513	560[a]	2.5	+9.2	107.0	526[a]	5.5	+2.5

[a]Each sample was diluted tenfold before measurement.

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accuracy for OXC and NOC ranged from −6.6% to +1.1% and from −11.7% to +2.5%, respectively. The performance results of this assay are satisfactory in terms of precision and accuracy.

Recovery of OXC and NOC from Sample Plasma

As shown in Table 1, the absolute recovery of OXC and NOC from human plasma ranged from 104.4% to 108.7% and from 102.9% to 107.0% for the assay range, respectively. The compounds were recovered quantitatively.

Stability of Assay Solutions

The results of the stability test are given in Table 2 for OXC and NOC in assay solutions. The percentages remaining were calculated by comparison of the measured value with the initial concentration. The concentrations of OXC and NOC remained unchanged over 6 days. OXC and NOC in the extracted samples were stable during 4°C storage in the autosampler at least for 6 days.

Stability of Plasma Samples

As shown in Table 3, OXC and NOC in human plasma samples subjected to three and five freeze–thaw cycles ranged from 89.8% to 105.5%. Thus, they were stable at five freeze–thaw cycles.

The results of the stability test of OXC and NOC in frozen plasma samples, presented in Table 4, show that both remained stable for at least 8 months in plasma samples stored at −20°C.

Table 2. Stability of OXC and NOC in assay solutions stored at 4°C ($n = 3$).

Compound	Initial conc. (ng/mL)	Days after storage	
		0	6
OXC	1.37	100.0	97.1
	42.4	100.0	102.6
NOC	2.64	100.0	99.2
	79.9	100.0	103.3

Note: Data represent mean percent remaining as value based on initial concentration.

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Table 3. Stability of OXC and NOC in human plasma under freeze–thaw cycles ($n = 3$).

Compound	Initial conc. (ng/mL)	Number of cycles		
		0	3	5
OXC	1.33	100.0	98.5	94.7
	58.1	100.0	95.5	89.8
	293[a]	100.0	96.9	91.9
NOC	2.54	100.0	105.5	102.8
	110	100.0	96.4	93.6
	544[a]	100.0	99.6	96.9

Note: Data represent mean percent remaining as value based on initial concentration.
[a]Each sample was diluted tenfold before measurement.

The results of the stability test for OXC and NOC in plasma samples at room temperature, given in Table 5, show that both remained stable for at least 48 hr in these samples over the assay range.

Stability of Blood Samples

Considering the storage conditions at clinical trial sites, the stability of OXC and NOC in whole blood samples was examined. The results of the stability test at 4°C, given in Table 6, show that the concentrations of OXC and NOC tended to increase because blood cells were hemolyzed with time and

Table 4. Stability of OXC and NOC in human plasma stored at −20°C ($n = 2$).

Compound	Initial conc. (ng/mL)	Months after storage	
		0	8
OXC	1.07	100.0	93.5
	5.27	100.0	93.0
	43.0	100.0	94.0
NOC	2.14	100.0	83.6
	10.6	100.0	94.1
	86.3	100.0	97.5

Note: Data represent mean percent remaining as value based on initial concentration.

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Table 5. Stability of OXC and NOC in human plasma stored at room temperature ($n = 3$).

Compound	Initial conc. (ng/mL)	Hours after storage	
		0	48
OXC	1.30	100.0	97.7
	52.3	100.0	104.2
	262[a]	100.0	104.2
NOC	2.59	100.0	106.6
	103	100.0	104.9
	513[a]	100.0	105.1

Note: Data represent mean percent remaining as value based on initial concentration.
[a]Each sample was diluted tenfold before measurement.

equilibrium transition between blood cell and plasma may occur. OXC and NOC remained stable for up to 5 days in whole blood samples stored at 4°C over the assay range.

The results of the stability test of OXC and NOC in whole blood samples at room temperature, listed in Table 7, are similar to those for whole blood stored at 4°C; the concentrations of OXC and NOC showed a tendency to increase with time. OXC and NOC seem to remain stable for up to 5 hr in whole blood samples stored at room temperature over the assay range.

Table 6. Stability of OXC and NOC in human whole blood stored at 4°C ($n = 3$).

Compound	Initial conc. (ng/mL)	Days after storage			
		0	2	5	7
OXC	1.15	100.0	100.0	104.3	100.0
	37.1	100.0	111.1	114.6	116.2
	189[a]	100.0	108.5	111.1	113.8
NOC	1.99	100.0	106.0	106.0	111.6
	69.6	100.0	105.6	107.9	109.1
	348[a]	100.0	104.3	106.9	108.9

Note: Data represent mean percent remaining as value based on initial concentration.
[a]Each sample was diluted tenfold before measurement.

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Table 7. Stability of OXC and NOC in human whole blood stored at room temperature ($n = 3$).

Compound	Initial conc. (ng/mL)	Hours after storage			
		0	5	24	48
OXC	1.11	100.0	94.6	100.0	107.2
	36.9	100.0	109.8	116.5	123.0
	195[a]	100.0	104.6	111.3	113.3
NOC	2.04	100.0	95.1	101.5	101.5
	70.3	100.0	101.6	108.0	113.5
	341[a]	100.0	102.6	112.9	112.9

Note: Data represent mean percent remaining as value based on initial concentration.
[a]Each sample was diluted tenfold before measurement.

Sample Throughput

For chromatographic analysis, all the objective compounds are eluted within approximately 10 min. The injection interval of the autosampler was set at 18 min.

Application of the Method

Clinical studies were carried out in which 5–40 mg of controlled-release tablets of OXC hydrochloride (OxyContin tablet) were administered orally to patients with cancer. The OXC and NOC levels over the period for all patients enrolled in this study were obtained and 366 samples were analyzed.

CONCLUSIONS

We established a simple, convenient and reliable method for the determination of OXC and NOC in human plasma. The method has adequate stability, robustness, selectivity and sensitivity. OXC and NOC are stable under its conditions. In view of the number of samples that can be analyzed, this method is suitable for routine analysis of OXC and NOC in human plasma samples for clinical studies over a wide assay range.

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REFERENCES

1. Kalso, E.; Vainio, A. Morphine and oxycodone hydrochloride in the management of cancer pain. Clin. Pharmacol. Ther. **1990**, *47*, 639–646.
2. Pöyhiä, R.; Olkkola, K.T.; Seppälä, T.; Kalso, E. The pharmacokinetics of oxycodone after intravenous injection in adults. Br. J. Clin. Pharmac. **1991**, *32*, 516–518.
3. Leow, K.P.; Smith, M.T.; Williams, B.; Cramond, T. Single-dose and steady-state pharmacokinetics and pharmacodynamics of oxycodone in patients with cancer. Clin. Pharmacol. Ther. **1992**, *52*, 487–495.
4. Pöyhiä, R.; Vainio, A.; Kalso, E. A review of oxycodone's clinical pharmacokinetics and pharmacodynamics. J. Pain Symptom Manage. **1993**, *8*, 63–67.
5. Beaver, W.T.; Wallenstein, S.L.; Rogers, A.; Houde, R.W. Analgesic studies of codeine and oxycodone in patients with cancer. II. Comparisons of intramuscular oxycodone with intramuscular morphine and codeine. J. Pharmacol. Exp. Ther. **1978**, *207*, 101–108.
6. Pöyhiä, R.; Seppälä, T.; Olkkola, K.T.; Kalso, E. The pharmacokinetics and metabolism of oxycodone after intramuscular and oral administration to healthy subjects. J. Clin. Pharmac. **1992**, *33*, 617–621.
7. Kaiko, R.F.; Benziger, D.P.; Fitzmartin, R.D.; Burke, B.E.; Reder, R.F.; Goldenheim, P.D. Pharmacokinetic–pharmacodynamic relationships of controlled-release oxycodone. Clin. Pharmacol. Ther. **1996**, *59*, 52–61.
8. Wey, A.B.; Thormann, W. Capillary electrophoresis and capillary electrophoresis-ion trap multiple-stage mass spectrometry for the differentiation and identification of oxycodone and its major metabolites in human urine. J. Chromatogr. B **2002**, *770*, 191–205.
9. Smith, M.T.; Watt, J.A.; Mapp, G.P.; Cramond, T. Quantitation of oxycodone in human plasma using high-performance liquid chromatography with electrochemical detection. Ther. Drug Monit. **1991**, *13*, 126–130.
10. Wright, A.W.E.; Lawrence, J.A.; Iu, M.; Cramond, T.; Smith, M.T. Solid-phase extraction method with high-performance liquid chromatography and electrochemical detection for the quantitative analysis of oxycodone in human plasma. J. Chromatogr. B **1998**, *712*, 169–175.
11. Caufield, W.V.; Stewart, J.T. Rapid determination of selected drugs of abuse in human plasma using a monolithic silica HPLC column and solid phase extraction. J. Liq. Chromatogr. Relat. Technol. **2002**, *25*, 2977–2998.
12. Kapil, R.P.; Padovani, P.K.; King, S.P.; Lam, G.N. Nanogram level quantitation of oxycodone in human plasma by capillary gas chromatography using nitrogenphosphorus selective detection. J. Chromatogr. **1992**, *577*, 283–287.

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13. Ropero-Miller, J.D.; Lambing, M.K.; Winecker, R.E. Simultaneous quantitation of opioids in blood by GC-EI-MS analysis following deproteination, detautomerization of keto analytes, solid-phase extraction, and trimethylsilyl derivatization. J. Anal. Toxicol. **2002**, *26*, 524–528.
14. Dawson, M.; Fryirs, B.; Kelly, T.; Keegan, J.; Mather, L.E. A rapid and sensitive high-performance liquid chromatography-electrospray ionization-triple quadrupole mass spectrometry method for the quantitation of oxycodone in human plasma. J. Chromatgr. Sci. **2002**, *40*, 40–44.
15. Knight, A.W.; Greenway, G.M. Relationship between structural attributes and observed electrogenerated chemiluminescence (ECL) activity of tertiary amines as potential analytes for the *tris*(2,2-bipyridine)ruthenium (II) ECL. Analyst **1996**, *121*, 101R–106R.
16. Knight, A.W. A review of recent trends in analytical applications of electrogenerated chemiluminescence. Trends Anal. Chem. **1999**, *18*, 47–62.
17. Gemba, Y.; Hashimoto, H.; Konishi, M. Simultaneous determination of oxycodone and noroxycodone in dog plasma and urine by HPLC, with post-column chemiluminescence detection using electrogenerated *tris*(2,2′-bipyridyl) ruthenium (III). J. Liq. Chromatogr. Relat. Technol. **2004**, *27*, 843–862.
18. Broglé, K.; Ornaf, R.M.; Wu, D.; Palermo, P.J. Peak fronting in reversed-phase high-performance liquid chromatography: a study of the chromatographic behavior of oxycodone hydrochloride. J. Pharm. Biomed. Anal. **1999**, *19*, 669–678.

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