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Sensitive UPLC–MS/MS Method for Oxycodone Quantification in Serum and Brain Tissue Homogenates: Application to an Interaction Study in Rats

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ABSTRACT

Objectives: An ultra-performance liquid chromatography tandem mass spectrometry (UPLC–MS/MS) assay has been developed and validated for the quantification of oxycodone in rat serum and brain tissue homogenates. **Methods:** A simple extraction method using methyl-t-butyl ether was used for sample extraction with oxycodone–D_3 as an internal standard (IS). A Symmetry® C_{18} column (100 mm x 4.6 mm, 5 µm) connected to a Phenomenex Luna® guard column (4 x 3 mm, 5 µm) was used for chromatographic separation. The mobile phase was composed of a mixture of acetonitrile and 0.1 % formic acid in water (pH = 2.7) (15:85, v/v). The flow rate was 0.4 mL/min, the total run time was 5 min, and the injection volume was 10 µL. The column and autosampler temperatures were maintained at 60 °C and 25 °C, respectively. **Results:** The calibration curve for oxycodone was linear over the range of 10–2000 ng/mL. Oxycodone extraction recovery from rat serum and brain samples ranged from 90.78–105.85 % and 98.42–104.38 % with relative standard deviation (RSD) values of 0.94–3.87 % and 1.04–2.82 %, respectively. The inter-day accuracy values ranged from 87.67–104.83 %, while the intra-day accuracy values ranged from 86.95–105.67 %. **Conclusion:** This method can be used for the quantification of oxycodone in samples obtained from preclinical animal studies and has great promise for applications in the quantification of oxycodone in human biological matrices.

***Keywords**: UPLC–MS/MS; Oxycodone; Rats; Serum; Brain*

INTRODUCTION

Fatal poisonings resulting from the abuse of opioid analgesics have increased exponentially in the United States from 1999 until now. The Drug Abuse Warning Network (DAWN) has reported that the predominant opioid analgesics that are abused are oxycodone, hydrocodone, and methadone[1]. Oxycodone is a semi-synthetic opioid agonist, acting on both mu- and kappa-receptors, derived from the naturally occurring opium (Thebaine) and is generally used to manage moderate to severe pain[2–4].

The analgesic properties of oxycodone have been proven to be one and a half times more potent than those of morphine among patients with postoperative and cancer-related pain[5]. In addition to its demonstrated

beneficial therapeutic use in pain management, oxycodone is considered the drug of choice and one of the most extensively available and abused opioids among drug addicts[6,7].

In a study performed in spring 2010, 56 % of students of two recovery high schools in Massachusetts indicated that oxycodone was their favourite prescription opioid type to abuse. Study participants attributed the low cost and availability of oxycodone in addition to the quality of the high obtained to their preference of its abuse[7]. Over 64,000 ICU department visits were due to the abuse of oxycodone in 2006, mostly coming from rural and western regions of the U.S. High rates of OxyContin® injection (an extended release oxycodone formulation) were reported among non-urban groups of prescription opioid users[8].

To enhance the euphoric effects or counteract sedation induced by opiates, drug addicts usually tend to administer various substances in combination. In a recent study, poly-drug abuse deaths were reported in 96.7 % of the cases. Multiple drug abuse was reported by toxicological testing with an average of 3.5 drugs abused, in which oxycodone was included in combination with other drugs, especially cocaine[9].

The Centers for Disease Control and Prevention reported that between 1999 and 2016, 630,000 people died from drug overdose. During 2017, there were more than 72,000 overdose deaths, including 49,000 involving opioids. In 2017, a total of 191 million opioid prescriptions were dispensed with a prescription rate of 58.5 prescriptions per 100 person[10]

Oxycodone has emerged as one of the most commonly abused substances in the past two decades, necessitating the development of a highly sensitive, precise and accurate method for its quantification in biological matrices to act as a reliable diagnostic tool for oxycodone abuse detection. Several different techniques have been used for the quantification of oxycodone alone or together with other opioids and commonly co-abused substances such as cocaine in various biological fluids, including blood, saliva, urine, hair, and meconium samples. These methods have included HPLC[11,12], gas chromatography coupled with mass spectrometry (GC/MS)[13,14], and liquid chromatography coupled with tandem mass spectrometry (LC/MS-MS)[15–30]. The reported methods have limitations, including the use of a large volume of organic solvent for extraction, such as 6 mL diethyl ether[11], 4 mL dichloromethane[12], 5 mL methanol[13], 5 mL chloroform/trifluoroethanol (10:1)[14], 4 mL n-butyl chloride: acetonitrile (4:1)[15], 6 mL ethyl acetate[16], 2 mL methanol and 2 mL ethyl acetate[27], 3 mL methanol[29,30], 4 mL methanol[19], and 2 mL ethyl acetate[20], as well as the use of relatively large sample volumes ranging from 1 to 2 mL of blood, plasma, and urine[11, 14, 15, 26, 29, 30].

Our objective was to develop a simple, reliable, sensitive and rapid LC/MS-MS method for the quantification of oxycodone in the presence of cocaine to overcome the drawbacks of the previously reported methods. We employed a simple and cost-effective sample preparation technique using a small volume of organic solvent (1 mL) and a small sample volume (500 µL) for the quantification of oxycodone in the presence of cocaine in rat serum and brain tissue samples. Our proposed method has been developed and validated according to the FDA guidance for bioanalytical method validation and has been successfully applied to quantify oxycodone in biological samples obtained from an oxycodone–cocaine interaction study.

MATERIAL AND METHODS

Chemicals and materials

Oxycodone hydrochloride and cocaine were purchased from Normaco, Inc. (Wilmington, DE, USA) and Sigma-Aldrich (St. Louis, MO, USA). Oxycodone–D_3 (the internal standard (IS)) was purchased from Cerilliant Corporation (Round Rock, TX, USA). All solvents were LC-MS grade; acetonitrile, methyl-t-butyl ether, formic acid, and water were purchased from Fisher Scientific (Fair Lawn, NJ, USA).

Male adult Sprague–Dawley rats were purchased from Harlan Laboratories (Frederick, MD). Blank serum from Sprague–Dawley rats was obtained from Valley Biomedical (Winchester, VA, USA). Blank brain homogenate was prepared by mixing fresh tissue obtained from untreated animals with a certain amount of saline (1:2, w/v) obtained from Baxter International Inc. (Deerfield, IL). The mixture was homogenized using a Fisher Scientific PowerGen Model 125 homogenizer (Fair Lawn, NJ). The samples were centrifuged using an Eppendorf® centrifuge 5417R (temperature controlled). The organic solvents were evaporated using a Multivap Nitrogen Evaporator.

Instruments

An Acquity ultra-performance liquid chromatography (UPLC) system (Waters Corporation; Milford, MA, USA) was used for chromatographic separations. Mass spectrometric detections were carried out using an Acquity tandem quadrupole mass spectrometer (Waters Corporation; Milford, MA, USA) operating in positive electrospray ionization (ESI) and multiple reaction monitoring (MRM) mode. Empower 3.0 software (Waters Corporation; Milford, MA USA) was used for data acquisition and processing.

Table 1. LC–MS/MS optimum parameters for quantification of oxycodone and oxycodone–D_3

Analyte	Q1 (m/z)	Q3 (m/z)	CV (V)	CE (eV)	Retention time (min)
Oxycodone	316.10	298.18	30	21	2.28
Oxycodone–D_3	319.16	301.18	35	19	2.27

Q1=Precursor ion; Q3=Product ion; CV=Cone voltage; CE=Collision energy

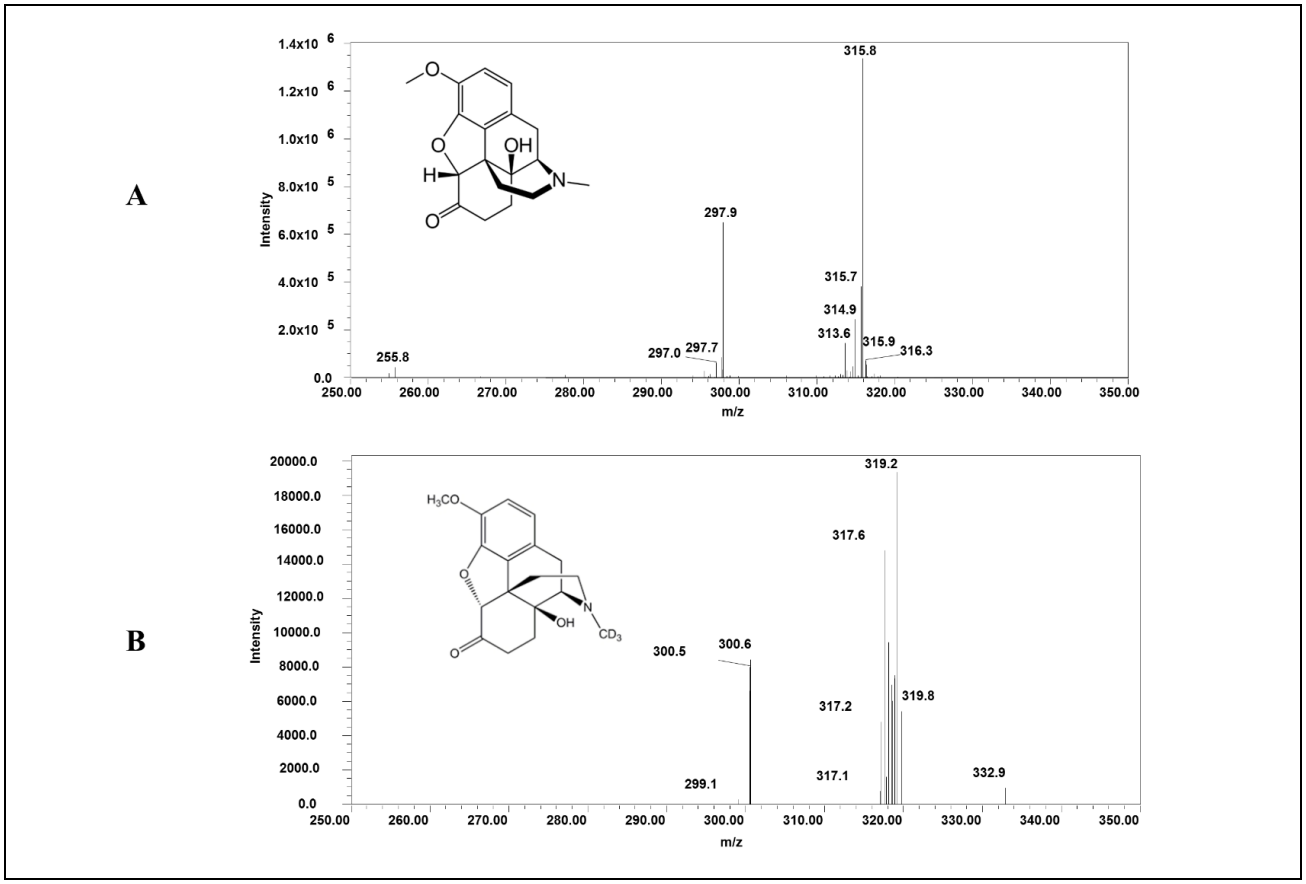

Figure 1. Full product ion scan of (a) oxycodone and (b) oxycodone–D_3.

Liquid chromatographic and mass spectrometric conditions

Chromatographic separations were achieved on a Waters® Symmetry C_{18} column (100 x 4.6 mm, 5 µm) connected to a Phenomenex Luna® security guard column (4 x 3 mm, 5 µm) (Phenomenex; Torrance, CA, USA) maintained at 60 °C. The mobile phase consisted of acetonitrile: 0.1 % formic acid (pH = 2.7) at 15:85 (v/v) and was used at a flow rate of 0.4 mL/min. The injection volume was 10 µL, and the total run time was 5 min. The mass spectrometric detection of oxycodone and its isotopically labelled IS (oxycodone-D_3) was conducted in positive ESI mode. Their MRM transitions were *m/z* 316.10 → 298.18 $[M + H]^+$ (oxycodone) and *m/z* 319.16 → 301.18 $[M + H]^+$ (oxycodone-D_3). The mass spectrometer was operated under the following parameters: nitrogen desolvation gas: 700 L/h, nitrogen cone gas: 50 L/h, source temperature: 150 °C, desolvation temperature: 500 °C, capillary voltage 3.2 kV, cone voltage: 35 V, extractor voltage: 3 V and RF lens voltage: 0.1 V.

Standard solutions and calibration curves

The oxycodone stock solution was prepared at a concentration of 1 mg/mL in methanol as the solvent, and the stock solution of its stable isotope-labelled IS (oxycodone–D_3) was dissolved in acetonitrile at a concentration of 100 µg/mL. The standard working solutions were prepared by diluting the stock solution with methanol. The internal standard working solution was diluted to a concentration of 500 ng/mL. All the stock and working solutions were stored at –80 °C.

The standards used to construct the calibration curve were prepared in serum or brain homogenate by spiking 450 µL of the thawed blank serum or brain tissue homogenate with a specific volume (50 µL) of the corresponding working solutions (10, 50, 100, 500, 1000, and 2000 ng/mL for oxycodone). Similarly, the quality control samples were freshly prepared from the working solutions at low, medium and high concentrations (30, 800 and 1500 ng/mL).

Sample preparation

Aliquots of the serum or brain homogenate (500 µL) were spiked with 10 µL of 500 ng/mL oxycodone–D_3. For liquid–liquid extractions, 1 mL of methyl-t-butyl ether was added to the sample, and the mixture was vortexed for 10 seconds at a high speed (2500 rpm) for 20 min and then centrifuged at 14,000 rpm for 30 min at 4 °C. The supernatant was transferred into a clean Eppendorf's tube and concentrated to dryness under nitrogen. The residue was then reconstituted mobile phase, and 10 µL of this solution was injected into the UPLC–MS/MS instrument for analysis.

Assay validation

Bioanalytical method validation was conducted according to the FDA guidelines, and the parameters of selectivity, linearity, range, accuracy, precision, recovery, and stability were determined [31]

Selectivity

The selectivity was assessed by comparing the chromatograms of six different lots of blank serum with another set of blank serum samples spiked with oxycodone at the LLOQ.

Linearity and range

A calibration curve was prepared using six oxycodone standards at concentrations ranging from 10 to 2000 ng/mL. The linearity was evaluated by plotting the oxycodone peak areas normalized with the IS peak (y) versus the oxycodone nominal serum/brain concentration (x). The calibration curve was fitted with a weighted ($1/y^2$) least square linear regression. The sensitivity of the method was calculated based on the LLOQ. Deviation from the nominal value for each calculated standard concentration did not exceed ± 15 %, while a deviation of ± 20 % was allowed at the LLOQ.

Accuracy and precision

The inter-day precision and accuracy were determined by analysing six replicates of each quality control sample on three consecutive days (between-run). The intra-day precision and accuracy were determined by analysing six replicates of the quality control samples on the same day (within-run). According to the FDA guidelines, the accuracy should be within ± 15 % of the nominal value. The precision is reported in terms of the % RSD, which should be within ± 15 %.

Recovery, matrix effects and carryover

The method recovery was demonstrated by comparing the peak areas of six replicate extractions of quality samples with the mean peak areas of extracted blank samples spiked after extraction. The matrix effects on the ionization of the analyte were evaluated by comparing the peak areas of the six post-extracted quality control samples with those prepared in methanol at the same concentration levels. The carryover was evaluated by injecting blank samples (n=3) after a QCH sample. Carryover into the blank sample should be less than 20 % of LLOQ.

Stability

Quality control samples at low and high levels were prepared and used to assess oxycodone stability under different conditions. They were stored at –80 °C for 24 h and then thawed to room temperature three times (three freeze and thaw cycles). Additionally, they were evaluated after being left at room temperature for 4 h and for 12 h in the autosampler. The long-term stability was assessed based on storage at –80 °C for 30 days. Additionally, stock solution stability was assessed by comparing a stock solution that had been stored for 60 days to a freshly prepared solution. For the stability to be acceptable, the deviation between measured values and nominal values should be within ± 15 %.

Application to an interaction pharmacokinetic study

The UPLC–MS/MS method was used to analyse samples from an interaction study of oxycodone and cocaine in rats. Adult male Sprague–Dawley rats weighing 250 to 300 g were used in the study. The protocol was approved by the IACUC (Institutional Animal Care and Use Committee) of the School of Pharmacy, University of Maryland, Baltimore. The rats were randomly divided into two groups with twenty-four rats per group. The first group was given saline (1 mL/kg, i.p.) twice daily. The other group was given oxycodone (5 mg/kg, i.p.) twice daily.

Table 2. Intra and inter-day precision and accuracy data for oxycodone in rat serum and brain samples

Nominal concentration (ng/mL)	Intra- day (n=6)			Inter-day (n =18)		
	Mean (ng/mL)	Accuracy (%)	RSD (%)	Mean (ng/mL)	Accuracy (%)	RSD (%)
Serum						
10	9.47	94.70	6.40	9.73	97.35	8.10
30	28.61	95.37	7.50	29.40	98.01	8.97
800	843.76	105.47	1.91	836.24	104.53	5.08
1500	1304.25	86.95	3.06	1315.05	87.67	2.37
Brain						
10	10.11	101.12	6.35	9.59	95.96	8.10
30	31.70	105.67	6.71	31.45	104.83	7.83
800	758.24	94.78	2.70	764.72	95.59	3.87
1500	1386.15	92.41	3.71	1362.00	90.80	4.61

RSD = Relative standard deviation

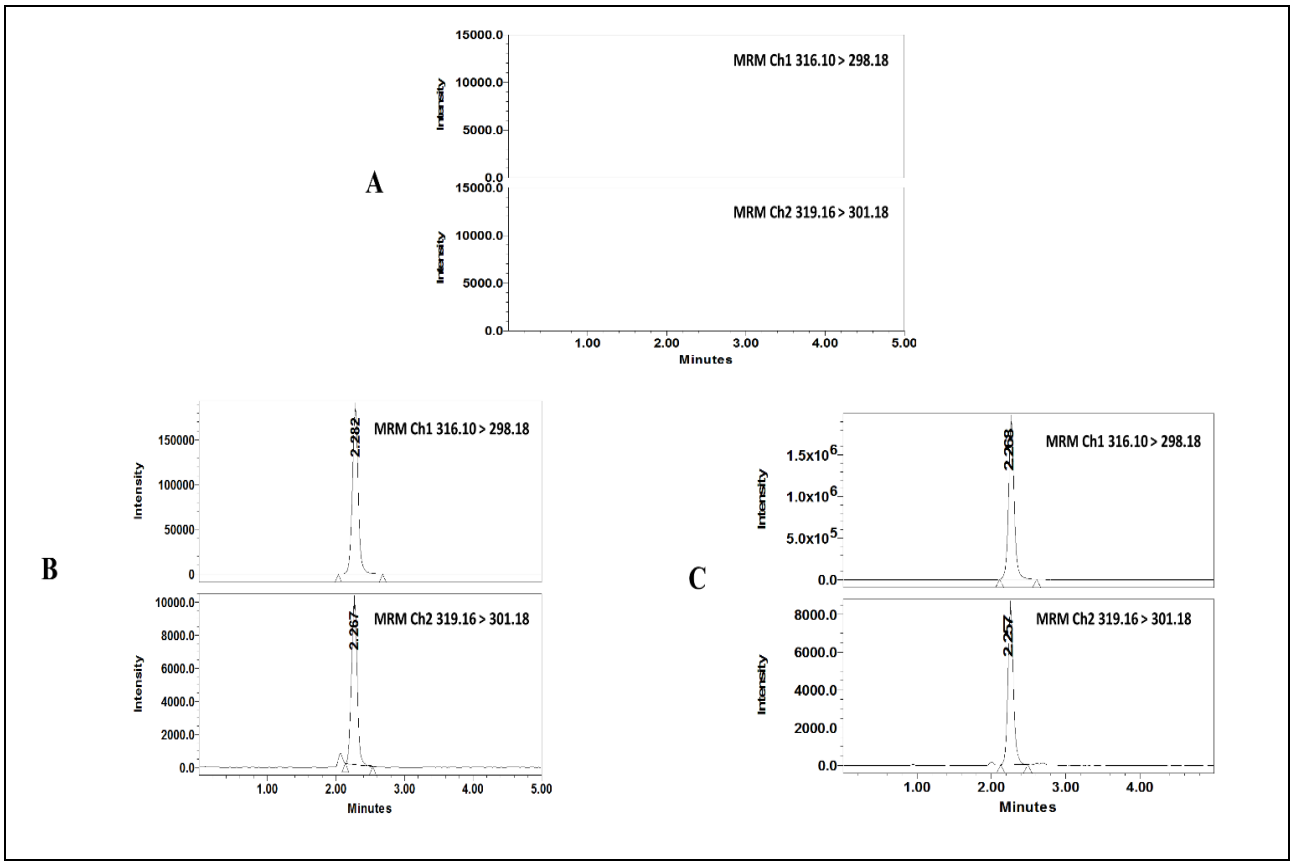

Figure 2. Representative MRM chromatograms of (a) blank rat serum,(b) rat serum spiked with oxycodone at the LLOQ (10 ng/mL) and (c) rat serum spiked with oxycodone at ULOQ (2000 ng/mL).

Table 3. Recovery and matrix effect for quantification of oxycodone in rat serum and brain samples

	Recovery Mean % (RSD) (n = 6)	Matrix effect Mean % (RSD) (n = 6)
Serum		
30	90.78 (0.94)	92.57 (0.93)
800	105.85 (2.19)	106.00 (6.57)
1500	98.85 (2.19)	101.64 (3.14)
Brain		
30	98.42 (2.02)	105.43 (1.83)
800	102.58 (1.04)	106.66 (1.72)
1500	104.38 (2.82)	101.04 (3.85)

This pre-treatment was performed for eight days to achieve a steady state. On day 9, following pre-treatment with saline or oxycodone, the rats were given cocaine (10 mg/kg, i.p.) and were sacrificed at 2, 5, 10, 15, 30, 60, or 120 min after cocaine administration. Blood and brain tissues were collected at the designated times. The blood samples were obtained by heart puncture using pre-heparinized syringes after euthanasia using the gradual fill method for CO_2 gas administration in a closed, commercially available cylinder with a pressure-reducing regulator and a flow metre. Blood samples were collected into tubes and were kept at room temperature for 30 min for clot formation. The samples were then centrifuged at 1300 *x* g for 20 min at 4 °C for serum collection and stored at -80 °C until analysis. Brain tissue was harvested, dry-blotted, weighed, homogenized with saline, and stored at -80 °C until analysis.

RESULTS AND DISCUSSION

According to the Centers for Disease Control and Prevention, in 2016, an estimated 2.2 million persons in the United States had received treatment to reduce illicit drug use, including prescription drug abuse[10]. Oxycodone has emerged as one of the most commonly abused substances in the past two decades, necessitating the development of highly sensitive, precise and accurate methods for its quantification in biological matrices to serve as reliable diagnostic tools for oxycodone abuse detection. Oxycodone abusers may co-administer cocaine to counteract the sedation effect of oxycodone.

The objective of this study was to develop an LC-MS/MS method for the quantification of oxycodone while overcoming the drawbacks of previously reported methods. The method validation was based on the FDA guidance for bioanalytical analysis[31]

Method development

Optimization of mass spectrometric parameters

Using an ESI source, we carried out a full scan of the oxycodone standard and IS in both positive and negative ionization modes by directly injecting the appropriate standard solution. Positive ionization mode yielded a more stable and higher response than was obtained in negative ionization mode. To ensure the reproducibility of the responses to oxycodone and the IS, we optimized the compounds and the instrument-specific mass spectrometric parameters.

In Q_1 scan mode, oxycodone and oxycodone–D_3 (IS) gave abundant singly charged protonated $[M+H]^+$ products with *m/z* 316.10 and 319.16 in product scan mode. The fragment ions of *m/z* 298.18 and 301.18 were the dominant fragment ions for oxycodone and oxycodone–D_3, respectively, and were thus used for quantification. The optimum MRM transitions for quantification were as follows: oxycodone (316.10 → 298.18) and oxycodone (D3) (319.16 → 301.18) as shown in **Table 1**. The full-scan product ion spectra for oxycodone and oxycodone–D_3 are shown in **Figure 1**.

Optimization of the chromatographic conditions

We optimized the chromatographic conditions to determine the most appropriate stationary phase and mobile phase to achieve good peak shape, resolution and sensitivity. We assessed different reversed-phase columns and flow rates to obtain the best retention time

Table 4. Summary of stability of oxycodone in rat serum and brain samples under different conditions

Nominal concentration (ng/mL)	30			1500		
	Mean (ng/mL)	Accuracy (%)	RSD (%)	Mean (ng/mL)	Accuracy (%)	RSD (%)
Serum						
Freeze-thaw	29.15	97.16	9.20	1324.20	88.28	2.56
Bench top	28.35	94.49	5.39	1338.30	89.22	2.37
Long term	29.28	97.60	7.15	1314.15	87.61	1.20
Processed sample	28.61	95.37	7.52	1304.25	86.95	3.06
Stock solution	29.13	97.11	9.54	1313.85	87.59	1.71
Brain						
Freeze-thaw	30.73	102.43	7.25	1345.80	89.72	4.49
Bench top	31.02	103.41	6.10	1351.95	90.13	1.45
Long term	30.76	102.52	6.67	1356.00	90.40	5.38
Processed sample	29.45	98.16	9.11	1367.25	91.15	6.68
Stock solution	29.99	99.97	7.20	1327.80	88.52	1.28

RSD = Relative standard deviation

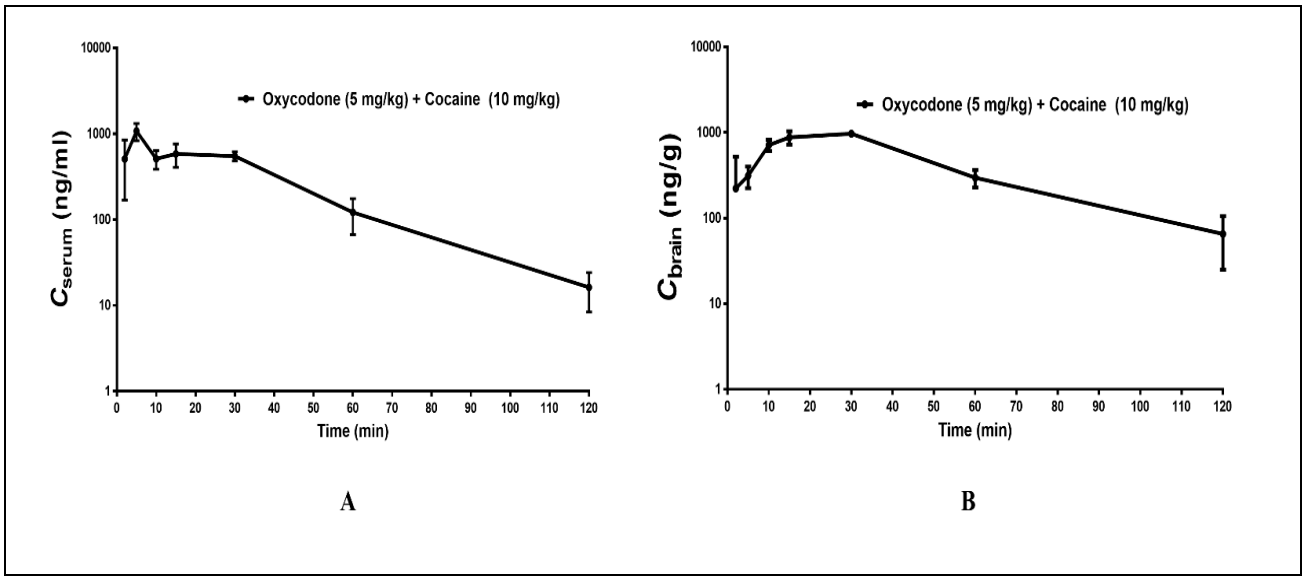

Figure 3. Oxycodone serum and brain concentrations vs time profiles after i.p. administration of oxycodone (5 mg/kg) in the presence of cocaine (10 mg/kg) to male Sprague–Dawley rats.

and peak shape for oxycodone and its IS. The following reversed-phase columns were assessed: Thermo Hypersil® C_{18} (150 mm x 4.6, 5 µm), Thermo Hypersil® C_8 (150 mm x 4.6, 5 µm) and Waters Symmetry® (100 mm x 4.6, 5 µm). We investigated different ratios of acetonitrile and water containing 0.1 % formic acid as the mobile phase.

Due to the polarity of oxycodone, we performed a rapid elution with a high proportion of organic solvent in the mobile phase. The retention time of oxycodone was 2.2 min at a ratio of 15:85 (v/v) acetonitrile: 0.1 % formic acid. However, interference with the oxycodone peak was observed when using both the C_{18} and C_8 Hypersil® columns. We later discovered that the interfering peak was due to the tendency of oxycodone to adhere to surfaces, leading to residual oxycodone on both the autosampler and the column itself, resulting in carryover. This carryover problem was solved by the addition of 0.1 % formic acid to both the strong and weak needle wash (up to 0.25 % formic acid), and the replacement of the water in both washes with acetonitrile. Additionally, we adjusted the column temperature to 60 °C. Indeed, we were able to successfully prevent carryover of the oxycodone into subsequent runs and obtain clean chromatograms with symmetric oxycodone and IS peaks.

A Waters Symmetry® C_{18} column (100 mm x 4.6, 5 µm) together with a Phenomenex Luna® security guard column (4 x 3 mm, 5 µm) yielded good retention of both oxycodone and its IS in addition to symmetric peaks and good sensitivity.

Optimization of the sample extraction procedure

Following the optimization of both the mass spectrometric parameters and the chromatographic conditions, we developed an effective sample extraction procedure to overcome and/or eliminate matrix interferences. We investigated both plasma and serum during the development and optimization of our sample extraction procedure. Extracting the analyte from the serum samples using methyl–t-butyl ether yielded cleaner chromatograms than were obtained following extraction from the plasma samples due to the relatively lower protein content in the serum samples. We investigated LLE techniques with two solvents. First, we tested ethyl acetate by preparing 3 standard curves and evaluating the extraction recovery. Due to the high polarity of ethyl acetate, phospholipids were extracted from the serum samples along with oxycodone, which led to unclean samples. Therefore, we tested methyl-t-butyl ether, which is relatively less polar than ethyl acetate and would not extract phospholipids from the serum samples, enhancing sample cleanliness and extraction recovery. Finally, dried samples were reconstituted in acetonitrile, which aided in the precipitation of any endogenous serum components and consequently led to ESI enhancement.

Assay validation

Selectivity

No interferences from endogenous serum components with oxycodone were observed in the analysis of six different lots of blank serum. The selectivity of the assay for oxycodone was confirmed in both blank serum and blank brain homogenate samples.

Linearity and range

The oxycodone assay was linear over the range of 10 to 2000 ng/mL. This parameter was evaluated based on the mean of three determinations at six concentration levels. Representative chromatograms for rat blank serum, blank serum spiked with oxycodone at the LLOQ (10 ng/mL) and blank plasma spiked with oxycodone at the ULOQ (2000 ng/mL) with oxycodone–D_3 as the IS are shown in **Figure 2**. Peak area ratios of oxycodone to its stable isotope-labelled IS (oxycodone–D_3) were plotted against their corresponding nominal concentrations to construct the calibration curves. The regression equation mean values were $(Y = 1.324\,x + 1.418, r^2 = 0.971)$ and $(Y = 0.1325\,x + 1.389, r^2 = 0.97)$ for oxycodone in serum and brain samples, respectively, where Y is the peak area ratio of oxycodone to the IS and x is the concentration of the analyte in ng/mL. We included zero and blank samples in our analysis to evaluate the reproducibility of our sample preparation procedure and verify the absence of any interferences. Our LLOQ for oxycodone was 10 ng/mL, which is the lowest concentration of the analyte to be quantitively determined with acceptable precision and accuracy, and a sample at this concentration was included in the evaluation of various method validation parameters. A deviation of ± 15 % from each calculated standard concentration from its nominal value was accepted, while ± 20 % deviation was only considered acceptable at the LLOQ.

Accuracy and precision

The intra-day and inter-day accuracy and precision results are presented in **Table 2**. The inter-day accuracy values of the assay were 87.67–104.53 % and 90.80–104.83 % with precisions of 2.37–8.97 % and 3.87–7.83 % for oxycodone in serum and brain samples, respectively. The intra-day accuracy values were 86.95–105.47 % and 92.41–105.67 % with RSDs of 1.91–7.50 % and 2.70–6.71 % for oxycodone in the serum and brain, respectively.

The obtained extraction recovery percentages were 90.78–105.85 % and 98.42–104.38 % with RSD values of 0.94–3.87 % and 1.04–2.82 % for oxycodone.

Table 5. Pharmacokinetic parameters of oxycodone in rat serum and brain tissue homogenate

Parameter	Oxycodone	
	Serum	Brain
$t_{1/2}$ (min)	18	24
C_{max} (ng/mL)	1076	972
T_{max} (min)	5	30
$AUC_{0 \rightarrow last}$ (min*ng/mL)	32288	56639
Cl (mL/(min*kg))	153	
AUC_{brain}/AUC_{serum} (mL/g)	1.8	

$t_{1/2}$: Half-life; C_{max}: maximum concentration; T_{max}: time to reach maximum concentration; AUC: Area under the curve; Cl: clearance.

in serum and brain samples, respectively. The recovery results indicated that methyl–t-butyl ether was suitable for the extraction of oxycodone from both serum and brain samples.

We investigated the matrix effects to evaluate any possible ionization enhancement or suppression caused by components in the matrix. Acceptable recoveries were obtained, which indicated the effectiveness of our sample extraction procedure in removing possible interferences from matrix components. The matrix effects were examined by comparing the mean peak areas of oxycodone at three concentrations (QCL, QCM and QCH) in extracted serum/brain samples to those obtained from clean standards at the same concentrations. The obtained recovery data ranged from 92.57–106.00 % and 101.04–106.66 % with precisions of 0.93–6.57 % and 1.72–3.85 % for serum and brain samples, respectively.

The recovery and matrix effects data for oxycodone at three concentrations (QCL, QCM and QCH) are shown in **Table 3**. We evaluated the carryover after injecting oxycodone at QCH and then analysing a blank serum/brain sample. No oxycodone was detected after repeating this procedure three times, which indicated the absence of any carryover.

Stability

We compared the obtained response from analysing QC samples at two concentration levels (QCL and QCH) subjected to freeze-thaw cycles to the responses of freshly prepared samples. The results demonstrated the good stability of our stock solution, and the determination of oxycodone in the QC samples was not affected by three freeze-thaw cycles. No oxycodone degradation was observed after thawing the frozen samples or after leaving them on the bench for 12 h. The QC samples remained stable after storage at –80 °C for 30 days. The quantification of oxycodone in the extracted samples was not affected by storage in an autosampler for 12 h. **Table 4** shows the stability results under different conditions.

Pharmacokinetic study

The rats were divided into two groups. The members of one group (n = 24) were pretreated with oxycodone twice daily for eight days before cocaine administration on day 9, and the other (control) group (n = 24) was pretreated with saline. The serum and brain samples obtained from the oxycodone-pretreated rats were analysed by LC-MS/MS for the quantification of oxycodone.

The pharmacokinetic analysis of oxycodone, as shown in **Table 5**, indicated that the serum C_{max} 1076 ng/mL compared to brain C_{max} 972 ng/mL. The clearance of oxycodone was 153 mL/min/kg while, the serum half-life of oxycodone was 18 min, but this was found to be 24 min in brain. The serum samples, the $AUC_{0 \rightarrow last}$ in the experimental groups decreased from 32288 min* ng/mL to 56639 min* ng/mL with brain samples. **Figure 3** shows the oxycodone (5 mg/kg) serum and brain concentration vs time profiles in the presence of cocaine (10 mg/kg).

CONCLUSION

A simple, sensitive and cost-effective LC/MS-MS method for the determination of oxycodone in rat serum and brain samples was developed and validated to support a preclinical pharmacokinetic study of the interactions between oxycodone and cocaine and to serve a reliable LC-MS/MS method for the quantification of oxycodone. The extraction method employed a simple, single-step process and resulted in a good sensitivity with an LLOQ of 10 ng/mL for oxycodone. The method has been applied for the quantification of oxycodone in rat serum and brain

samples obtained from our PK study of the DDIs between oxycodone and cocaine.

Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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