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Extraction and quantitation of carfentanil and naltrexone in goat plasma with liquid chromatography–mass spectrometry

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Abstract

This method is the first analytical method for the detection and quantitation of carfentanil and naltrexone at clinically relevant concentrations using liquid chromatography–mass spectrometry. Samples were alkalinized with 100 μl of 1 *M* NaOH and extracted 2× with 2 ml of toluene. The extractions were combined and dried under N_2 at 40 °C in a H_2O bath. Chromatography was performed using a Zirchrom PBD column and a mobile phase of 30:70 acetonitrile/10 mM ammonium acetate and 0.1 mM citrate (pH=4.4) at a flow rate of 0.3 ml/min. The lower limit of quantitation was 8.5 pg/ml for carfentanil and 0.21 ng/ml for naltrexone.

Keywords: Carfentanil; Naltrexone

1. Introduction

Carfentanil (CARF) is the most potent opioid agonist currently in use. It is 20× more potent than fentanyl [1], and is approved by the United States Food and Drug Administration for immobilization of free-ranging or confined members of the family Cervidae (i.e., white-tailed deer, elk, and moose). Since its development in 1975, CARF has become the drug of choice for immobilization of a wide variety of non-domestic mammals [1,2], because it allows for rapid and reliable induction of anesthesia with small volumes of CARF in a diverse range of species [3]. Carfentanil is a synthetic derivative of fentanyl (Fig. 1). In most situations, CARF anesthesia is reversed using the antagonist naltrexone (NLT) [1].

Several studies of CARF have been reported in both domestic [4,5] and non-domestic animals [6,7] but the pharmacokinetics and tissue residues of CARF are unknown. Due to the extremely potent nature of CARF, very low concentrations of CARF are believed to be achieved in the blood and it has not been previously possible to accurately measure the low concentrations of CARF in plasma. While there is a semi-commercial ELISA assay available, the limit of quantitation is 1 ng/ml [8]. The use of liquid chromatography–mass spectrometry (LC–MS) will allow for the determination of plasma levels of CARF prior to and following reversal of

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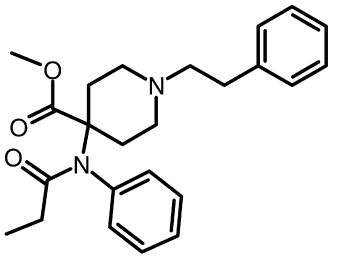
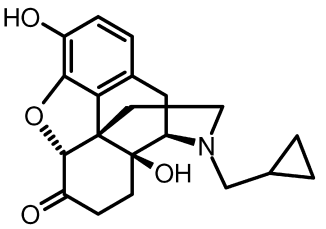
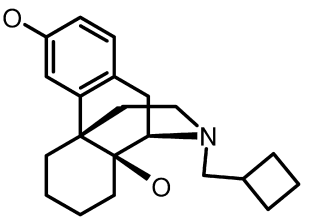


Carfentanil



Naltrexone



Butorphanol (IS)

Fig. 1. The structures of carfentanil, naltrexone, and butorphanol.

anesthesia with greater sensitivity and confidence. The purpose of this project was to develop an analytical method to quantitate the plasma concentrations of CARF and NLT using a single extraction method. This will assist with providing information on the pharmacokinetics of these compounds.

2. Experimental

2.1. Chemicals

The toluene, 1 *M* NaOH, and citrate were certified grade (Fisher Scientific, Pittsburg, PA, USA). Carfentanil (purity ≥99%) was obtained as a 3-mg/ml solution from Wildlife Laboratories, Fort Collins, CO, USA. Naltrexone (purity ≥99%) and the internal standard butorphanol (purity ≥99%) were obtained from Sigma, St. Louis, MO, USA. Water, isopropanol, acetonitrile, ammonium acetate, and acetic acid were HPLC grade (Fisher Scientific, Pittsburg, PA, USA).

2.2. Instrumentation

The LC–MS system (ThermoFinnigan, San Jose, CA, USA) comprised of a P4000 narrow-bore quaternary pump with a vacuum degasser, AS3000 auto sampler, UV6000 photo-diode array detector, and a LCQ_{duo} quadrupole ion-trap mass spectrometer. MS instrument parameters: ionization source–ESI, electrospray ionization, positive; spray voltage: 4.0 kV; sheath gas flow-rate: 50 arbitrary units; auxiliary gas flow-rate: 32 arbitrary units; capillary voltage: 30 V; capillary temperature: 235 °C; tube lens offset: −5.0 V; lens voltage: −38 V; multipole 1 offset: −1.5 V; multipole 2 offset: −4.5 V; MS–quantitation, single ion monitoring (SIM), ions (*m*/*z*); CARF–395.2; NLT–342.2; I.S.–Butorphanol–328.2; MS–method setup; segment 1, event 1–SIM 342.2 (±0.5) *m*/*z*; segment 1, event 2–data dependent scan, parent mass 342.2 (±0.5) *m*/*z*, normalized collision energy 30%, minimum signal 1×10^4; segment 2, event 1–SIM 395.2 (±0.5) *m*/*z* and 328.2 (±0.5) *m*/*z*; segment 2, event 2–data dependent scan, parent mass 395.2 (±0.5) *m*/*z*, normalized collision energy 30%, minimum signal 1×10^4; all of the instrumental parameters were optimized for CARF on the MS. The data system was the Xcalibur software suite (ThermoQuest, San Jose, CA, USA). Chromatography was performed as follows: column–Zirchrom PBD; 50×2.1 mm, 3 μm (Zirchrom Separations, Anoka, MN, USA); mobile phase–30:70 acetonitrile (ACN)/10 mM ammonium acetate and 0.1 mM citrate (pH=4.4); flow-rate–0.3 ml/min; retention time–CARF: ~2.7 min, NLT: ~1.7 (IS–Butorphanol: ~2.8 min).

The citrate was added to the mobile phase to reduce tailing. A concentration of 0.1 mM was found to reduce tailing while not compromising the mass spectrometry. Fentanyl has a pK_a of 8.4. No pK_a is reported for CARF but was assumed to be similar to fentanyl. The other opioids are reported to have pK_a's from 7 to 9 [9]. The mobile phase pH of 4.4 was at least 3 units below the estimated pK_a of

CARF and produced sufficient ionized compound for detection.

2.3. Preparation of standards and QCs

Stock solutions of CARF and NLT in mobile phase were made. The stock solutions were used to make solutions for the standard curve and QC samples in goat plasma. The volume of stock solution added to the goat plasma for both the standard curves and the QCs was ≤10% of the total volume. Standards, in goat plasma, were made fresh each day that samples were extracted. The goat plasma QC samples were made in bulk and aliquoted into 1-ml samples and stored at −20 °C until used. For assay validation, five QC samples each at three different concentrations were randomly selected. For the assay, two QC samples at each concentration were selected at random and extracted with the standard curve and unknown samples.

2.4. Recovery

To make samples for testing recovery, 100-μl aliquots of the appropriate standard curve stock solution were put into vials for detection on the LC–MS system. A 100-μl aliquot of 10× stock solution is equal to 100 μl aliquot added to make 1 ml goat plasma sample extracted and reconstituted in 100 μl mobile phase.

2.5. Extraction method

The extraction method was adapted from Martens-Lobenhoffer method for sufentanil [10]. The samples, standards, and QCs extracted were 1-ml aliquots placed in 13×100-mm glass test tubes (Fisher Scientific, Pittsburg, PA, USA). To each tube, 100 μl of internal standard (butorphanol) was added and vortexed. Then, to each tube 100 μl of 1-*M* NaOH, 200 μl of isopropanol, and 2 ml of toluene were added in order. The tubes were capped and vortexed for 1 min twice over a 40-min period. The tubes were then centrifuged for 10 min at 1000 *g*. The supernatant was transferred to a new 15-ml centrifuge tube, then each tube of plasma had 200 μl of isopropanol and 2 ml of toluene added. They were again vortexed and centrifuged as described above. The supernatants from both extractions were combined and then dried under N_2 in a 40 °C H_2O bath. The samples were then reconstituted with 100 μl of mobile phase, vortexed for 1 min, transferred to an auto sampler vial, and 50 μl was injected onto the LC–MS system.

Table 1
Recoveries for carfentanil and naltrexone from goat plasma

Carfentanil		Naltrexone	
pg/ml	% Recovery	ng/ml	% Recovery
10	22	0.25	49
25	67	1	75
100	82	5	90
500	105	10	92
1000	94	50	86
5000	103	100	96
10000	106	150	102
15000	84		
20000	100		

2.6. Quantitation

Peak area ratio of analyte:IS was used for both CARF and NLT quantitation. Standard curve ranges

Table 2
Mean and standard error (S.E.) values for slope, *y*-intercept, and R^2 for each standard curve

	Slope	S.E.	Intercept	S.E.	R^2	S.E.
Carfentanil 10–1000 pg/ml	0.00333	0.000146	0.000986	0.00318	0.9968	0.0002914
Carfentanil 1000–20000 pg/ml	0.0302	0.00759	0.361	0.0650	0.9900	0.0004449
Naltrexone 0.25–5 ng/ml	0.826	0.0382	−0.0821	0.00963	0.9895	0.0008279
Naltrexone 5–150 ng/ml	0.570	0.0388	0.801	0.109	0.9907	0.0004344

were: CARF low–10–1000 pg/ml; CARF high–1000–20000 pg/ml; NLT low–0.25–5 ng/ml; NLT high–5–150 ng/ml. Concentration vs. peak area ratio was plotted and a linear regression equation obtained for quantitation. QC levels were: CARF low–50, 250, and 750 pg/ml; CARF high–2500, 12500, and 17500 pg/ml; NLT low–0.5 and 2.5 ng/ml; NLT high–7.5, 25, and 125 ng/ml.

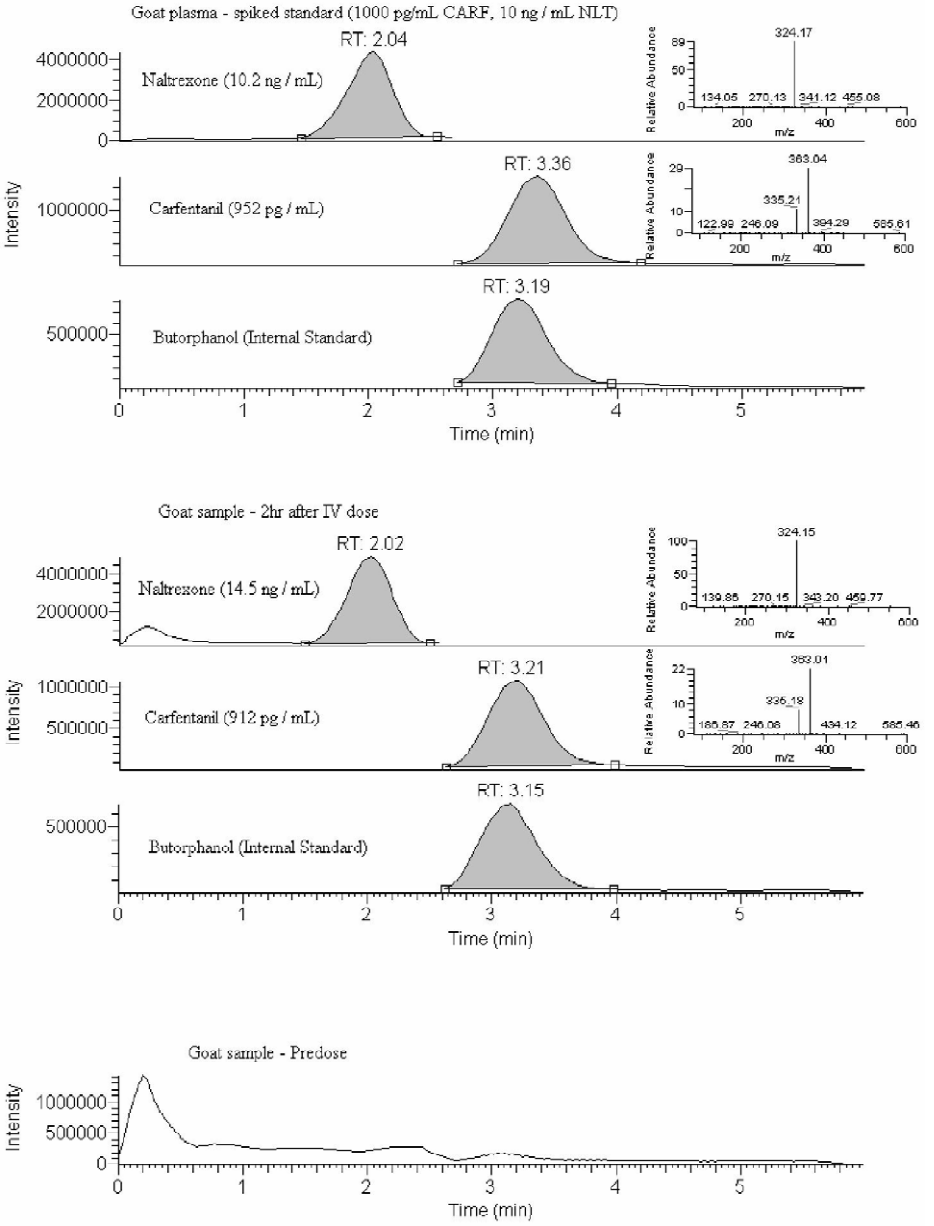


Fig. 2. Separation of carfentanil, naltrexone, and the internal standard butorphanol following extraction from goat plasma.

3. Results and discussion

The CARF assay validated with an intra-day ($n = 5$ samples QC) accuracy of $\leq \pm 8.4\%$ of intended; precision of $\leq \pm 14.7\%$, and recovery of $>80\%$ across the range of the standard curves, with the exception of concentrations ≤ 25 pg/ml, which ranged from 20 to 70% (Table 1). For NLT, intra-day ($n = 5$ samples QC) accuracy was $\leq \pm 12.6\%$; precision of $\leq \pm 9.4\%$; and recovery of $>80\%$ across the range of the standard curves with the exception of the 1-ng/ml value, which was 75% and the 0.25-ng/ml with 49% (Table 1). Inter-day (10 days with two samples per QC) accuracy and precision for CARF was $\leq \pm 9.5$ and $\leq \pm 6.2\%$, respectively; and $\leq \pm 10.3$ and $\leq \pm 6.6\%$ for NLT. A daily assay run was rejected if more than two of the six QCs for a given standard curve were $> \pm 15\%$ of their intended value or if both QCs at a single concentration were $> \pm 15\%$ of their intended concentration, the run was reassayed with fresh extractions. The lower limit of quantitation for CARF was 8.5 pg/ml and 0.21 ng/ml for NALT. This is 15% below the lowest standard curve concentration for each analyte. The mean and standard error values for the slope, y-intercept, and R^2 are reported in Table 2 for each standard curve.

Separation of CARF, NLT, and the I.S. (butorphanol) following extraction from goat plasma is shown in Fig. 2. The ability to quantitate CARF and NLT in a single sample with one extraction procedure was accomplished in this project. We are also able, for the first time, to detect and quantitate CARF at pharmacologically relevant plasma concentrations, two orders of magnitude lower than previously reported [8]. This method provides adequate sensitivity to detect CARF in the plasma of goats for up to 48 h after administration of 40 μg/kg intramuscularly (data not shown). The ability to detect and quantitate CARF and NLT with a single extraction dramatically decreases the time and money needed to perform sample analysis, especially since both drugs are commonly used concurrently in zoological medicine.

The NLT assay reported here is more sensitive than other HPLC methods [11–14] by at least one order of magnitude. Even when compared to a HPLC method with similar sensitivity, this assay required half the amount of sample [15]. This method allows for the simultaneous quantitation of CARF and NLT in plasma to support pharmacokinetic and pharmacodynamics studies in zoological species. The pharmacokinetic and pharmacodynamic studies will explore the cause or causes of renarcotization in zoological species.

Acknowledgements

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