USAMRICD-TR-22-01

Intramuscular Pharmacokinetics of Naltrexone in the African Green Monkey (Chlorocebus aethiops sabeus)

Jeffrey L. Langston
Mark C. Moffett
Jennifer R. Makar
Katherine N. Bullock
M. Ross Pennington
Todd M. Myers

January 2022

US Army Medical Research Institute of Chemical Defense
8350 Ricketts Point Road
Aberdeen Proving Ground, MD 21010-5400

an element of the

US Army Medical Research and Development Command

DISPOSITION INSTRUCTIONS:

Destroy this report when no longer needed. Do not return to the originator.

DISCLAIMERS:

The views expressed in this technical report are those of the author(s) and do not reflect the official policy of the Department of Army, Department of Defense, or the U.S. Government.

This work was supported by the Defense Threat Reduction Agency (DTRA), Advanced and Emerging Threats Division, Chemical Medical Countermeasures Team.

The experimental protocol was approved by the Animal Care and Use Committee at the United States Army Medical Research Institute of Chemical Defense, and all procedures were conducted in accordance with the principles stated in the Guide for the Care and Use of Laboratory Animals and the Animal Welfare Act of 1966 (P.L. 89-544), as amended.

The use of trade names does not constitute an official endorsement or approval of the use of such commercial hardware or software. This document may not be cited for purposes of advertisement.

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	**2. REPORT TYPE**	**3. DATES COVERED** *(From - To)*
12-01-2022	Technical	10 January 2018 to 6 June 2018

4. TITLE AND SUBTITLE
Intramuscular Pharmacokinetics of Naltrexone in the African Green Monkey (Chlorocebus aethiops sabeus)

5a. CONTRACT NUMBER

5b. GRANT NUMBER
CB3950

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Langston, Jeffrey L., Moffett, Mark C., Makar, Jennifer R., Bullock, Katherine N., Pennington, M. Ross, Myers, Todd M.

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

US Army Medical Research Institute of Chemical Defense
ATTN: FCMR-CDR-PS
8350 Ricketts Point Road
Aberdeen Proving Ground, MD 21010-5400

8. PERFORMING ORGANIZATION REPORT NUMBER
USAMRICD-TR-22-01

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

Defense Threat Reduction Agency
8725 John J. Kingman Road STOP 6201
Fort Belvoir, VA 22060-6201

10. SPONSOR/MONITOR'S ACRONYM(S)
DTRA

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for public release; distribution unlimited.

13. SUPPLEMENTARY NOTES
This work was supported by the Defense Threat Reduction Agency (DTRA), Advanced and Emerging Threats Division, Chemical Medical Countermeasures Team.

14. ABSTRACT

- The pharmacokinetics of intramuscular naltrexone human equivalent doses (HEDs) of 2, 10, and 20 mg were evaluated in adult male African green monkeys.
- Strong dose-linearity was observed between the injected naltrexone dose and plasma concentration maximum (C_{max}).
- Absorption was rapid, with time to maximum concentration (T_{max}) approximating 14 minutes for the 10 and 20 mg HEDs.
- Intramuscular administration of naltrexone in this laboratory non-human primate model exhibited orderly and predictable plasma kinetics.
- The inclusion of 10 and 20 mg HEDs allows for confident predictions of plasma levels at a bolus intramuscular dose above those commonly administered but potentially required for severe opioid exposure.

15. SUBJECT TERMS
Opioid, rescue therapy, emergency medicine, fentanyl

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			Todd M. Myers
UNCLASSIFIED	UNCLASSIFIED	UNCLASSIFIED	UNLIMITED	14	**19b. TELEPHONE NUMBER** *(include area code)* 410-436-8380

EXECUTIVE SUMMARY

- The pharmacokinetics of intramuscular naltrexone human equivalent doses (HEDs) of 2, 10, and 20 mg were evaluated in adult male African green monkeys.
- Strong dose-linearity was observed between the injected naltrexone dose and plasma concentration maximum (C_{max}).
- Absorption was rapid, with time to maximum concentration (T_{max}) approximating 14 minutes for the 10 and 20 mg HEDs.
- Intramuscular administration of naltrexone in this laboratory non-human primate model exhibited orderly and predictable plasma kinetics.
- The inclusion of 10 and 20 mg HEDs allows for confident predictions of plasma levels at a bolus intramuscular dose above those commonly administered but potentially required for severe opioid exposure.

INTRODUCTION

Naltrexone is an opioid receptor antagonist with activity at the μ-, κ-, and δ-opioid receptors (Toll et al., 1998). Naltrexone is more potent and has greater affinity for μ-opioid receptors than does naloxone, but is approximately equipotent to nalmefene (Emmerson et al., 1994; France et al., 1990; France and Gerak, 1994; Toll et al., 1998). Naltrexone has been approved for human use in multiple forms for decades. However, few publications detail the intramuscular pharmacokinetics of the immediate release formulations in humans or non-human primates. One report detailed the intravenous and oral pharmacokinetics of naltrexone in female rhesus macaques (Reuning et al., 1989). In that report, the absolute bioavailability of oral naltrexone (10 mg/kg) was 3.6%, and the terminal half-life following a one-minute IV infusion of naltrexone (10 mg/kg) was 13.74 (7.81; SD) hours. In another study by the same author (Reuning et al., 1979), the terminal half-life of IV naltrexone in six rhesus monkeys was reported to be 7.8 hours. In humans, the terminal half-life of naltrexone has been reported to be approximately 11 hours following intravenous administration and approximately 9.5 hours following oral administration (Licko, 1981).

The present study characterized the intramuscular pharmacokinetics of naltrexone hydrochloride in plasma using adult male African green monkeys. Three of four animals completed the entire pharmacokinetic time course characterization (from 2.5 minutes to 24 hours after drug administration) for each of three intramuscular naltrexone doses (65, 323, and 646 μg/kg body weight) equivalent to 2, 10, and 20 mg HEDs (as indicated in Table 1; c.f. FDA (2005)) and overlapping with the doses previously reported under separate studies evaluating naloxone's pharmacokinetics, behavioral safety, and efficacy in different animals of the same species, sex, and age (Langston et al., 2019; Langston et al., 2018).

MATERIAL AND METHODS

Subjects

Four experimentally experienced and trained adult male African green monkeys (*Chlorocebus aethiops sabeus*) (5.68-6.50 kg, mean 5.88 kg) of Caribbean origin were individually housed in stainless steel squeeze-back cages (with an effective area equal to ~ 61 cm W X 71 cm D X 86 cm H). The colony was maintained at 21 ± 2 ºC with a relative humidity of 50% ± 15% on a 12 hr light/dark cycle (lights on at 0600). Daily allotted food (Certified Primate Diet 5048, Purina Mills, Inc., St. Louis, MO, and fresh fruit and vegetables) was controlled to maintain healthy body weights, and water was available *ad libitum*. On drug administration and training days, the food ration was provided approximately 20 minutes after the 320-minute blood sample was collected. The experimental protocol was approved by the Animal Care and Use Committee at the United States Army Medical Research Institute of Chemical Defense (USAMRICD), and all procedures were conducted in accordance with the principles stated in the Guide for the Care and Use of Laboratory Animals and the Animal Welfare Act of 1966 (P.L. 89-

544), as amended. The USAMRICD is a research facility fully accredited by the AAALAC International.

Materials

Naltrexone HCl ((5α)-17-(Cyclopropylmethyl)-4,5-epoxy-3,14-dihydroxymorphinan-6-one hydrochloride) was obtained from Sigma-Aldrich (St. Louis, MO; ≥98% purity; N3136). The naltrexone salt was added to sterile physiological saline (0.9%) and passed through a 0.1 µm filter into a sterile vial. Stock solutions of naltrexone (up to 8.5 mg/mL) were made on a weekly basis and kept at 4 °C. On the day of experimentation, an aliquot of naltrexone was obtained from the stock solution and diluted to the desired concentration (1-8.5 mg/mL) to keep injection volumes at or below 0.50 mL (range of 0.37 to 0.47 mL). Drug concentrations and doses are based on the hydrochloride salt form of naltrexone.

Table 1. Naltrexone doses administered to the nonhuman primates and the corresponding human equivalent doses based on the FDA's body surface area scaling (FDA, 2005) and the reported absolute oral bioavailability of naltrexone of 3.6% in rhesus monkeys (Reuning et al., 1989).

Nonhuman Primate Dose (µg/kg)	Human Equivalent Dose (mg)	Human Oral Dose (mg)
64.63	2	40
323.15	10	200
646.30	20	400

Methods

Animals were placed in a primate restraint chair for blood collection and drug administration. Each naltrexone injection was administered into the lateral thigh muscle using a standard 25 gauge 5/8" needle and a 1 mL syringe. Blood samples were taken from the saphenous vein at prescribed post-administration time points of 2.5, 5, 10, 20, 40, 80, 160, 320, and 1440 minutes using a 21-25 gauge needle or catheter. Each sample was collected into a heparinized syringe, dispensed into a heparinized micro-centrifuge tube, and centrifuged at 10K RPM for 5 minutes at 4 °C. The supernatant (plasma) was carefully removed via transfer pipette and dispensed into a separate blank micro-centrifuge tube, flash frozen in a bath of dry ice and ethanol, and placed on dry ice until transfer to a -80 °C freezer. Plasma samples were stored at -80 °C until analysis.

An LC-MS/MS assay was developed for the analysis of naltrexone-exposed African green monkey plasma samples. The developed assay was validated using blank, heparinized African green monkey plasma (BioIVT, Chestertown, MD, USA) to prepare calibration curves and quality control samples. Plasma was spiked at 400 ng/mL with standardized naltrexone (1.0 mg/mL naltrexone, Cerilliant, Round Rock, TX, USA) and serially diluted with plasma to produce the following concentrations, which served as calibrators: 100, 25, 6.25, 1.56, and 0.391, ng/mL.

Isotopically labeled naltrexone (100 µg/mL naltrexone-D_3, Cerilliant, Round Rock, TX, USA) was spiked into each sample to produce a final concentration of 5 ng/mL in each sample. The assay was validated according to the FDA (2018) guidelines regarding bioanalytical method development. Calibration curves were generated in duplicate and analyzed in triplicate, and a total of 6 sets of calibration curves were prepared over non-consecutive days (five inter-day and 1 intra-day). Quality control (QC) samples were prepared at 100, 10 and 1.0 ng/mL. QC samples were used to determine intra- and inter-day variability. Quantification of the QC samples was accomplished by running a calibration curve on each day. A linear least squares analysis with a 1/y weighting scheme was used to calculate the calibration parameters. The precision (%CV) was calculated using the formula %CV = (SD/mean) x 100%, and the accuracy (%error) was calculated using the formula % error = ((calculated concentration – actual concentration)/actual concentration) x 100%. Precision and accuracy were below 15% for all validation samples and QCs.

Prior to processing, plasma samples were stored at -80 °C. Samples were thawed, and 200 µL was transferred to clean microcentrifuge tubes. Isotopically labeled naltrexone (100 µg/mL naltrexone-D_3, Cerilliant, Round Rock, TX, USA) was spiked into each sample to produce a final concentration of 5 ng/mL in each sample. A calibration curve was prepared each day that samples were processed. All calibrators, QCs and samples were extracted by solid-phase extraction (SPE) using Oasis 1 cc HLB cartridges with 30 mg sorbent (Waters Corporation, Milford, MA). The SPE procedure was as follows: 1) Wash with 2 mL methanol; 2) Wash with 2 mL water with 20 mM ammonium formate; 3) Load 100 µL sample; 4) Wash with 2 mL water with 20 mM ammonium formate; and 5) Elute with 2 mL methanol containing 0.2% formic acid. The eluent for all calibrators, QCs and samples was evaporated under a dry nitrogen stream at 40 °C. Samples were reconstituted in 90 µL of 10% methanol in 0.1% formic acid in water. Extraction was performed in duplicate, and the replicates were analyzed via LC-MS/MS in triplicate.

Liquid chromatography was performed using an Agilent 1290 Infinity liquid chromatograph (Agilent Technologies, Santa Clara, CA). Separation was performed on an Halo C18 column (2.7 µm, 2.1 mm x 50 mm) (Advanced Materials Technology, Wilmington, DE) with a chromatographic ramp with mobile phase B = 0.2% formic acid in methanol and mobile phase A = 0.2% formic acid, consisting of the following schedule: 0 min → 3min (10% mobile phase A → 95% mobile phase A), 3 min → 4 min (95% mobile phase A), 4.0 min → 4.1 min (95% mobile phase A → 10% mobile phase A), 4.1 →7 minutes (10% mobile phase A). The flow rate was 500 µL/min and an injection volume of 5 µL was used. A retention time of 1.1 min was observed.

Tandem mass spectrometry was accomplished using a Sciex 6500 QTrap triple quadrupole mass spectrometer (Sciex, Ottawa, CA). It was operated in electrospray mode using multiple reaction monitoring (MRM). The ion source temperature was 700 °C. Capillary voltage was +5500V, curtain gas was 30 and the collision-assisted dissociation gas was medium. Ion source gas 1 and 2 were 50 and 70. Declustering potential was 50V and entrance potential was 10V.

For naltrexone, the quantifier ion transition was 342.2 Da to 270.0 Da with collision energy of 36eV and collision exit potential of 9V, while the qualifier ion transition was 342 Da to 212.2 Da with collision energy of 28eV and collision exit potential of 7V. For naltrexone-D_3, the quantifier ion transition was 345.2 Da to 270.0 Da with collision energy of 38eV and collision exit potential of 14V, while the qualifier ion transition was 342 Da to 212.2 Da with collision energy of 29eV and collision exit potential of 9V. Peak areas were integrated using Analyst software (Sciex, Ottawa, Ontario).

Plasma concentration-time data for intramuscular naltrexone were fit using nonlinear least squares regression and adequately described by a one-compartment model with first-order absorption and elimination (Gibaldi and Perrier, 1982). The differential equations governing the PK model are

$$\frac{dX_a}{dt} = -k_a X_a \quad (1)$$

$$\frac{dX_c}{dt} = k_a X_a - K X_c \quad (2)$$

where k_a is the absorption rate and K is the elimination rate. Additional pharmacokinetic parameters (e.g., $t_{½}$ k_a, $t_{½}$ ke, C_{max}, $t_{max,}$ etc.) were estimated according to the methods of Gibaldi and Perrier (1982). Individual plasma concentration-time data were fit using the PKfit (v.1.3.8) package for R (v.3.2.5; R Core Team, 2013; Vienna, Austria). The linearity of C_{max} and $AUC_{0\text{-}inf}$ were determined via linear regression. Non-compartmental analyses (NCA), which do not rely on assumptions about body compartments and tend to provide better replicability across analysts, were conducted using the PKNCA (v.0.8.1) package for R (Denney et al., 2015).

RESULTS

The intramuscular pharmacokinetic data were best described by a one-compartment model with first-order absorption and elimination. The mean plasma concentrations across the first 5 hours and 20 minutes of sampling for all three doses of naltrexone are presented in Figure 1. Individual subject plasma concentration-time data are presented in Appendix 1.

Pharmacokinetic estimates were determined for each animal, and the group means are shown in Table 2. Naltrexone absorption was rapid, with measurable concentrations occurring 2.5 min following IM administration. Time to maximal plasma concentrations (T_{max}) appeared to be independent of dose and occurred at approximately 16, 14, and 14 minutes for the 2, 10, and 20 mg HEDs, respectively. Predictably, C_{max} and $AUC_{0\text{-}inf}$ both increased linearly with dose ($R^2 > 0.94$ for both). Elimination half-life appeared to decrease with increasing doses of naltrexone, but not greatly. Neither apparent volume of distribution (V/F) nor apparent clearance (Cl/F) demonstrated dose-related trends.

Pharmacokinetic parameter estimates from non-compartmental analyses of the plasma concentration-time data following intramuscular naltrexone HCl are presented in Table 3. Both analyses provided similar estimates of T_{max}, C_{max}, and $AUC_{0\text{-}inf}$, but differences were observed for elimination half-life ($T_{1/2}$). The NCA $T_{1/2}$ for the two lowest doses of naltrexone was 1.08 hours each, whereas that of the highest dose was estimated to be 3.78 hours.

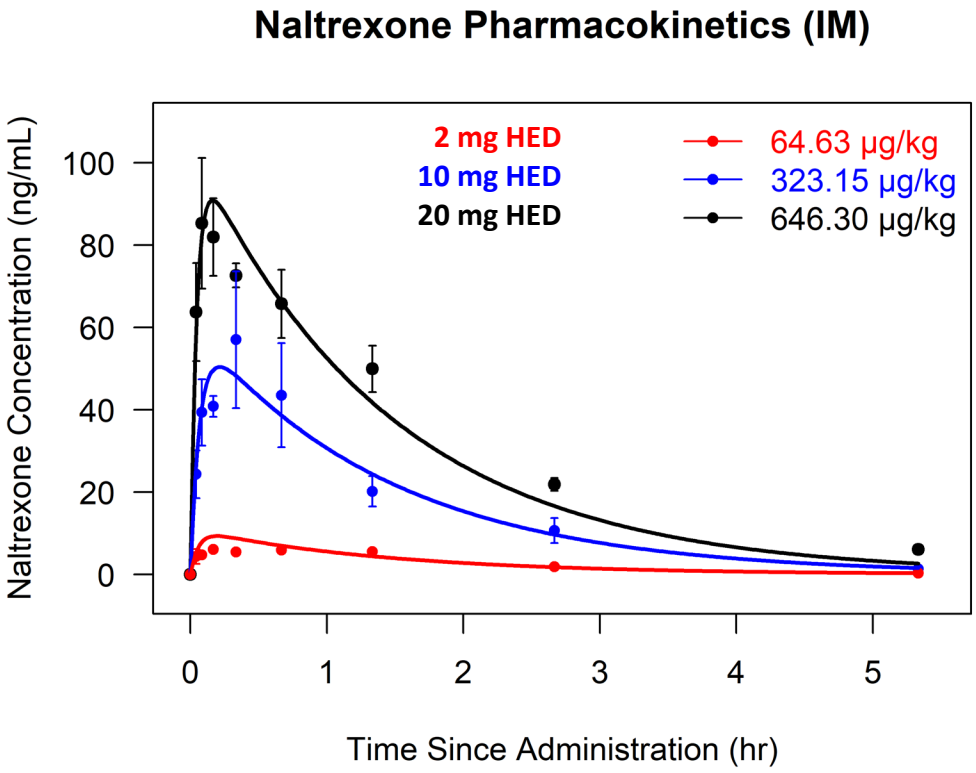


Figure 1. Pharmacokinetics of intramuscular naltrexone in adult male African green monkeys. Points are mean plasma concentrations of n=3-4 animals, vertical bars are ± SEM. Solid lines are the predicted plasma concentrations derived from the average fitted parameter estimates from the one-compartment model.

Table 2. Mean (SD) of pharmacokinetic parameter estimates from one-compartment model for naltrexone HCl following intramuscular administration to African green monkeys (n=3-4).

Parameter	64.63 µg/kg (2 mg HED)	323.15 µg/kg (10 mg HED)	646.30 µg/kg (20 mg HED)
T_{max} (hr)	0.26 (0.25)	0.23 (0.14)	0.24 (0.20)
$t_{½}$ ka (hr)	0.06 (0.07)	0.09 (0.10)	0.13 (0.18)
C_{max} (ng/mL)	7.31 (0.56)	65.05 (27.63)	94.91 (19.06)
$t_{½}$ ke (hr)	1.50 (0.11)	0.80 (0.34)	0.84 (0.52)
$AUC_{0\text{-}inf}$ (µg.hr/L)	15.07 (1.53)	75.16 (11.00)	206.90 (26.96)
Cl/F (L/hr/kg)	3.85 (0.38)	4.38 (0.70)	4.58 (0.63)
V/F (L/kg)	8.33 (1.31)	5.10 (2.35)	5.25 (2.73)

Table 3. Pharmacokinetic parameter estimates for intramuscular naltrexone HCl in African green monkeys (n=3-4) from non-compartmental analyses

Parameter	64.63 µg/kg (2 mg HED)	323.15 µg/kg (10 mg HED)	646.30 µg/kg (20 mg HED)
C_{max} (ng/mL)[†]	7.30 (7.60)	61.4 (39.2)	93.7 (19.4)
T_{max} (hr)[‡]	0.167 (0.0417, 0.667)	0.208 (0.0833, 0.333)	0.167 (0.0833, 0.667)
$T_{1/2}$ (hr)[§]	1.08 (0.127)	1.08 (0.0399)	3.78 (0.693)
$AUC_{0\text{-}inf}$ (ug.hr/L)[†]	15.0 (10.4)	74.6 (15.5)	206 (12.7)

[†] Geometric mean (geometric CV). [‡] Median (min, max). [§] Arithmetic mean (std. dev.)

DISCUSSION

We evaluated the pharmacokinetic profile of intramuscular naltrexone at human equivalent doses of 2, 10, and 20 mg in adult male African green monkeys. Strong dose-linearity was observed between the injected naltrexone dose and maximal plasma concentration (C_{max}), as well as in AUC. Absorption was rapid, with time to maximum concentration (T_{max}) approximating 14 minutes for the 10 and 20 mg HEDs, and approximating 16 minutes for the 2 mg HED. Intramuscular administration of naltrexone in this laboratory non-human primate model exhibited orderly and predictable plasma kinetics that appear largely comparable to available human data following SC administration of naltrexone (Wall et al., 1984). The authors reported a terminal half-life of the parent compound of 1.68 hr. The same authors (Wall et al., 1981) reported terminal plasma half-life values of (unconjugated) naltrexone to be 2.7 hr and 8.9 hr following IV and oral administration, respectively. The prescription oral formulation (c.f. REVIA package insert; Full Prescribing Information; Reference ID: 3383348) has a reported terminal half-life of 4 hr for the parent compound.

Data from the limited animal studies of naltrexone pharmacokinetics reported terminal plasma half-lives between 7.8 and 13.74 hr (Reuning et al., 1989; Reuning et al., 1979). The differences in pharmacokinetic parameters (e.g., terminal plasma half-life) between these

animal studies and the human studies reported above may be due primarily to the pharmacokinetic model used to describe the data (i.e., two-compartment open model vs. three-compartment open model).

Compared to previous studies of naltrexone pharmacokinetics conducted using rhesus monkeys, the data from the present study indicate that naltrexone has a relatively short terminal plasma half-life in the African green monkey. Non-compartmental analysis revealed a dose dependency in terminal plasma half-life, in that the terminal half-life of the 20 mg HED in the African green monkey was 3.5-fold longer than that of the 2 mg and 10 mg HEDs. There are several potential explanations for this, including a saturable elimination pathway. Alternatively, plasma concentration-time data from the highest dose of naltrexone may require a higher order compartmental model (e.g., a two-compartment open model with first-order absorption and elimination) to adequately characterize the elimination rate constants.

The pharmacokinetics of IM naltrexone appear to be more favorable than those of naloxone for the treatment of long-acting opioids. Comparison of the non-compartmental pharmacokinetic analysis parameters from the present investigation with those of a previously conducted study of naloxone's IM pharmacokinetics in this laboratory with the same species (Langston et al., 2019) indicates that the elimination half-life of the 10 mg HED of naltrexone is approximately 30% longer than that of an equivalent dose of naloxone (10 mg HED). The longer half-life of naltrexone combined with its greater affinity for the μ-receptor (Emmerson et al., 1994; France et al., 1990; Raynor et al., 1994; Raynor et al., 1995; Toll et al., 1998) may yield better therapeutic and/or prophylactic efficacy against ultra-potent opioids such as carfentanil.

REFERENCES

Denney WS, Duvvuri S and Buckeridge C (2015) Simple, automatic noncompartmental analysis: The PKNCA R package. *Journal of Pharmacokinetics and Pharmacodynamics* **42**:65.

Emmerson PJ, Liu MR, Woods JH and Medzihradsky F (1994) Binding affinity and selectivity of opioids at mu, delta and kappa receptors in monkey brain membranes. *J Pharmacol Exp Ther* **271**:1630-1637.

FDA (2005) *Guidance for Industry: Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers.*, U.S. Department of Health and Human Services, Silver Spring, MD.

FDA (2018) *Guidance for Industry: Bioanalytical Method Validation*, U.S. Department of Health and Human Services, Silver Spring, MD.

France CP, de Costa BR, Jacobson AE, Rice KC and Woods JH (1990) Apparent affinity of opioid antagonists in morphine-treated rhesus monkeys discriminating between saline and naltrexone. *J Pharmacol Exp Ther* **252**:600-604.

France CP and Gerak LR (1994) Behavioral effects of 6-methylene naltrexone (nalmefene) in rhesus monkeys. *J Pharmacol Exp Ther* **270**:992-999.

Gibaldi M and Perrier D (1982) *Pharmacokinetics*, New York: Marcel Dekker.

Langston JL, Makar JM, Bullock KN, Pennington MR and Myers TM (2019) Intramuscular Pharmacokinetics of Naloxone in the African Green Monkey (Chlorocebus aethiops sabeus). *US Government Technical Report* **USAMRICD-TR-19-01**. Aberdeen Proving Ground, Maryland: U.S. Army Medical Research Institute of Chemical Defense. AD1076228

Langston JL, Moffett MC, Makar JM, Burgan BM and Myers TM (2018) Carfentanil Toxicity in the African Green Monkey: Therapeutic Efficacy of Naloxone. *US Government Technical Report* **USAMRICD-TR-18-03**. Aberdeen Proving Ground, Maryland: U.S. Army Medical Research Institute of Chemical Defense. AD1057869

Licko V (1981) Overview of human pharmacokinetics of naltrexone. *NIDA Res Monogr* **28**:161-171.

Raynor K, Kong H, Chen Y, Yasuda K, Yu L, Bell GI and Reisine T (1994) Pharmacological characterization of the cloned kappa-, delta-, and mu-opioid receptors. *Mol Pharmacol* **45**:330-334.

Raynor K, Kong H, Mestek A, Bye LS, Tian M, Liu J, Yu L and Reisine T (1995) Characterization of the cloned human mu opioid receptor. *J Pharmacol Exp Ther* **272**:423-428.

Reuning RH, Ashcraft SB, Wiley JN and Morrison BE (1989) Disposition and pharmacokinetics of naltrexone after intravenous and oral administration in rhesus monkeys. *Drug Metab Dispos* **17**:583-589.

Reuning RH, Batra VK, Ludden TM, Jao MY, Morrison BE, McCarthy DA, Harrigan SE, Ashcraft SB, Sams RA, Bathala MS, Staubus AE and Malspeis L (1979) Plasma naltrexone kinetics after intravenous bolus administration in dogs and monkeys. *J Pharm Sci* **68**:411-416.

Toll L, Berzetei-Gurske IP, Polgar WE, Brandt SR, Adapa ID, Rodriguez L, Schwartz RW, Haggart D, O'Brien A, White A, Kennedy JM, Craymer K, Farrington L and Auh JS (1998) Standard binding and functional assays related to medications development division testing for potential cocaine and opiate narcotic treatment medications. *NIDA Res Monogr* **178**:440-466.

Wall ME, Brine DR and Perez-Reyes M (1981) Metabolism and disposition of naltrexone in man after oral and intravenous administration. *Drug Metab Dispos* **9**:369-375.

Wall ME, Perez-Reyes M, Brine DR and Cook CE (1984) Naltrexone disposition in man after subcutaneous administration. *Drug Metab Dispos* **12**:677-682.

APPENDIX A

Table A-1. Naltrexone plasma concentrations (ng/mL) following IM administration of 64.63 µg/kg naltrexone HCl.

Time (h)	Subject 1	Subject 2	Subject 3	Subject 4
0	0	0	0	nd
0.04	2.344466	2.863625	7.913738	nd
0.08	5.111352	3.9154	5.280523	nd
0.16	7.211264	4.941694	6.168638	nd
0.33	6.502861	5.260404	4.55445	nd
0.67	5.839267	6.808183	5.230712	nd
1.33	5.120152	6.599602	4.914633	nd
2.67	2.541747	1.595067	1.611384	nd
5.33	0.289092	0.570282	0	nd
24	0	0	0	nd

nd: not determined

Table A-2. Naltrexone plasma concentrations (ng/mL) following IM administration of 323.15 µg/kg naltrexone HCl.

Time (h)	Subject 1	Subject 2	Subject 3	Subject 4
0	0	0	0	0
0.04	31.89508	15.21155	36.50387	13.7452
0.08	45.20715	23.00192	59.13991	30.13644
0.16	42.90766	41.37154	45.35356	33.67975
0.33	31.91675	50.27994	40.29462	105.5812
0.67	23.72654	30.96487	39.12069	80.28144
1.33	21.38773	24.9012	25.01543	9.410995
2.67	5.917365	10.13756	7.233113	5.604585
5.33	1.295205	2.067706	1.844429	19.31784
24	0	0	0	0

Table A-3. Naltrexone plasma concentrations (ng/mL) following IM administration of 646.30 µg/kg naltrexone HCl.

Time (h)	Subject 1	Subject 2	Subject 3	Subject 4
0	0	0	0	nd
0.04	50.35406	53.46147	87.49375	nd
0.08	72.6517	66.34997	116.8255	nd
0.16	85.7162	64.08244	96.01199	nd
0.33	69.69556	78.45091	69.72729	nd
0.67	55.88277	82.18592	59.16622	nd
1.33	51.84901	58.65542	39.2939	nd
2.67	19.53931	24.84909	21.22612	nd
5.33	6.55143	5.783431	5.904593	nd
24	0.123132	0.946258	0.489555	nd

nd: not determined

APPENDIX B

SIGMA-ALDRICH®

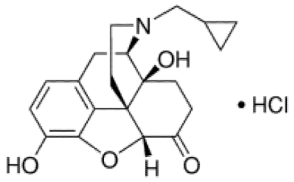
sigma-aldrich.com

3050 Spruce Street, Saint Louis, MO 63103, USA
Website: www.sigmaaldrich.com
Email USA: techserv@sial.com
Outside USA: eurtechserv@sial.com

Product Specification

Product Name:
Naltrexone hydrochloride

Product Number:	**N3136**
CAS Number:	16676-29-2
MDL:	MFCD00069324
Formula:	C20H23NO4 · HCl
Formula Weight:	377.86 g/mol
Storage Temperature:	2 - 8 ℃



TEST	Specification
Appearance (Color)	White to Off-White
Appearance (Form)	Powder
Solubility (Color)	Colorless to Faint Yellow
Solubility (Turbidity) 50 mg/ml, H2O	Clear to Very Slightly Hazy
Water (by Karl Fischer)	≤ 11 %
Carbon (anhydrous)	62.0 - 65.2 %
Nitrogen (anhydrous)	3.4 - 4.0 %
Purity (HPLC)	≥ 99 %
Recommended Retest Period 2 years	----------

Specification: PRD.1.ZQ5.10000031188