# METHOCARBAMOL THRESHOLD STUDY

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# RMTC Methocarbamol Study

- Introduction
  - Sponsor: Racing Medication and Testing Consortium
  - Title: Pharmacokinetics and Withdrawal Time Estimates for Methocarbamol after Single Dose Intravenous Administration and Multiple Dose Oral Administration to Thoroughbred Horses
  - Purpose: To determine the pharmacokinetics of methocarbamol and to estimate the withdrawal time of methocarbamol after single intravenous and multiple dose oral administration of methocarbamol at a clinically relevant dose to athletically conditioned Thoroughbred horses
  - Lead investigator: Dr. Richard Sams
  - Co-investigator: Dr. Scott D. Stanley
  - Preliminary Data: RMTC pilot study conducted in 2007 at UF and UC Davis

# RMTC Methocarbamol Study

- Animal Studies
  - Study Center: UF Pharmacokinetics Laboratory in the Equine Performance Laboratory at the University of Florida
  - Study Director: Dr. Patrick Colahan
  - Animal care and use protocol: approved by University of Florida IACUC
  - Facilities: Inspected twice annually by university IACUC team
  - Subjects: Thoroughbred horses housed at UF College of Veterinary Medicine facilities
  - Number of subjects: 20 (6 for pharmacokinetics studies)
  - Conditioning: Treadmill exercise three days per week except on dose administration days – able to run one mile in two minutes under conditions required for participation in study
  - Feed and water: Available at all times on study days

# Methocarbamol Study

- Withdrawal Study
  - Subjects:
    - Gender: Geldings (11) and mares (9)
    - Weight: 468-605 kg
    - Age: 5-10 years
  - Samples
    - Samples were collected before and at 24, 48, and 72 hours after IV dose administration
- Pharmacokinetic Study – Intravenous bolus dose
  - Subjects:
    - Gender: Geldings (6) and mares (0); subset of the subjects in withdrawal study
    - Weight: 505-560 kg
    - Age: 7-10 years
  - Samples
    - Samples were collected before and from 0.08 through 12 hours after dose administration and at 24, 48, and 72 hours after dose administration

# RMTC Methocarbamol Study

- Drug Administration Phase – IV Study
  - Drug: Methocarbamol
  - Source: Wedgewood Pharmacy, compounding pharmacy
  - Formulation: Solution for injection – 100 mg/mL
  - Dose: 15 mg/kg of body weight
  - Route: Intravenous
  - Frequency: Once
  - Sample collections: individual venipuncture at each collection point into partially-evacuated glass tubes containing Li heparin
  - Sample handling and storage: Plasma was harvested and stored at -70°C until analyzed (less than two months)

# Methocarbamol Study

- Pharmacokinetic Study – Repeat dose oral administration
  - Subjects:
    - Gender: Geldings (6) and mares (0); subset of the subjects in withdrawal study
    - Weight: 505-560 kg
    - Age: 7-10 years
  - Samples
    - Samples were collected before and from 0.08 through 12 hours after first dose administration, immediately before each subsequent dose administration, and from 0.08 through 16 hours and at 24, 48, and 72 hours after last dose administration.

# RMTC Methocarbamol Study

- Drug Administration Phase – Oral Study
  - Drug: Methocarbamol
  - Source: Webster Pharmacy
  - Formulation: Tablet – 500 mg
  - Dose: 5000 mg
  - Route: Oral
  - Frequency: Every 12 hours for five doses
  - Sample collections: individual venipuncture at each collection point into partially-evacuated glass tubes containing Li heparin
  - Sample handling and storage: Plasma was harvested and stored at -70°C until analyzed (less than two months)

# RMTC Methocarbamol Study

- Method Development and Validation Phase
  - Method Development Laboratory: Florida Racing Laboratory at the University of Florida
  - Method Development Phase Director: Dr. Richard Sams
  - Analyst: Marc Rumpler
  - Reference: US FDA Guidance Document for Method Validation
  - Method: Liquid-liquid extraction followed by liquid chromatographic-mass spectral analysis of methocarbamol and guaifenesin using stable isotope labeled analogues of the analytes as internal standards.
  - Standards:
    - Methocarbamol: United States Pharmacopeia and Sigma-Aldrich
    - Methocarbamol-$d_4$: Frontier Biopharm
    - Guaifenesin: Sigma-Aldrich
    - Guaifenesin-$d_3$: C/D/N Isotopes

# RMTC Methocarbamol Study

- Analytical Phase
  - Analytical Laboratory: Florida Racing Laboratory at the University of Florida
  - Analytical Phase Director: Dr. Richard Sams
  - Analyst: Marc Rumpler
  - Method: Liquid-liquid extraction followed by liquid chromatographic-mass spectral analysis of methocarbamol and guaifenesin using stable isotope labeled analogues of the analytes as internal standards
  - Calibrators: Nine calibrators (0.5, 1, 5, 10, 20, 50, 100, 200, 500 ng/mL) in matrix-matched control plasma in duplicate; prepared fresh on day of analysis. Calibrators contained methocarbamol and guaifenesin.
  - Controls: Five positive control samples (1, 25, 75, 250, 400 ng/mL) in duplicate prepared before start of sample analyses and stored under same conditions as study samples. Control samples contained methocarbamol and guaifenesin.
  - Samples: Samples were analyzed in duplicate. Samples containing methocarbamol at concentrations greater than 500 ng/mL were diluted with plasma before analysis.

# Methocarbamol Study

- Analytical Conditions
  - Column
    - Type: Acquity™ UPLC HSS T3 C18 Analytical Column
    - Dimension: 2.1 mm × 50 mm
    - Particle size: 1.8 µm
    - Temperature: 38 °C
  - Guard Column
    - Type: Acquity™ UPLC HSS T3 Vanguard Pre-column
    - Dimension: 2.1 mm x 5 mm
    - Particle size: 1.8 µm
  - Mobile Phase
    - Mobile phase A: 0.1% formic acid in water
    - Mobile phase B: 0.1% formic acid in methanol
    - Flow rate: 250 µL/min
  - Injection Volume: 20 µL

# SRM Acquisition Parameters

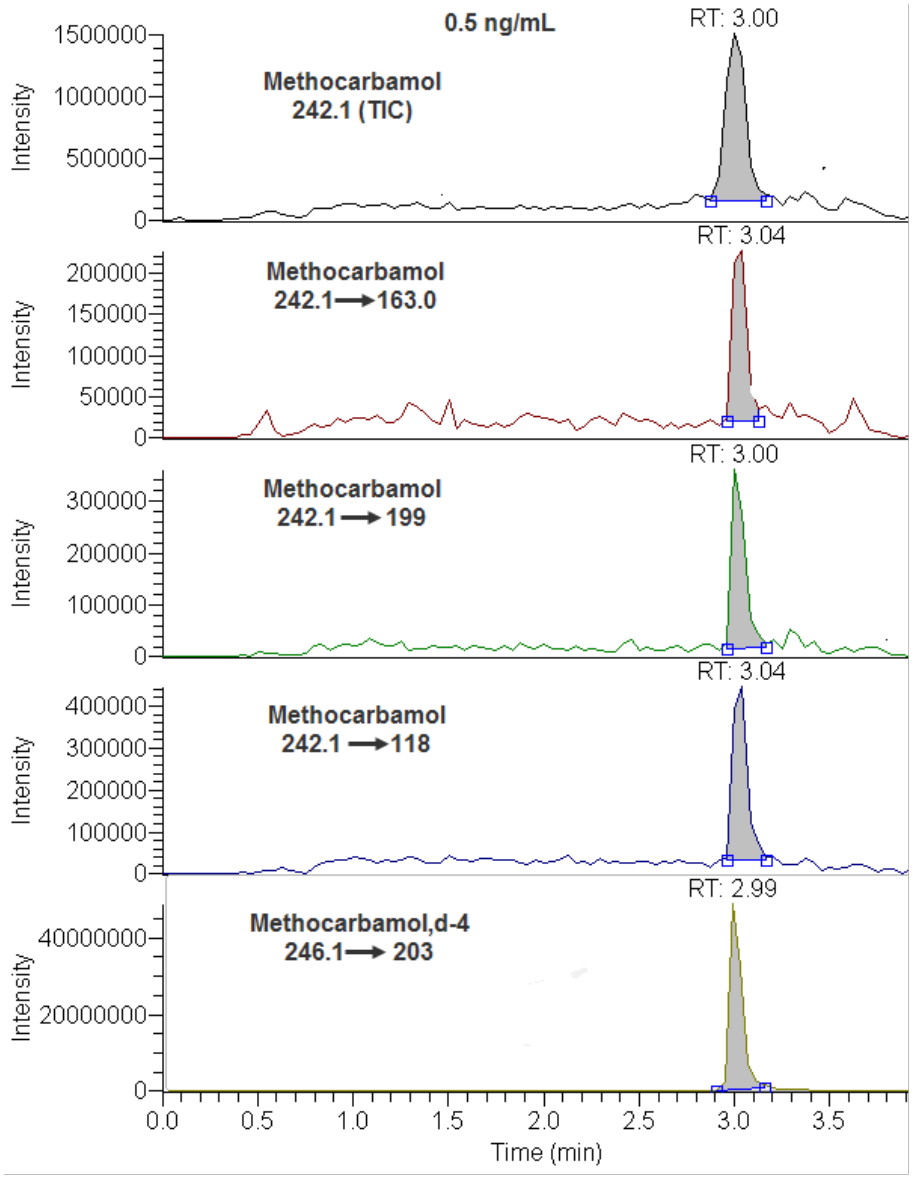
SRM acquisition parameters for methocarbamol and guaifenesin and their deuterated analogues.

| Analyte | Precursor Mass | Product Ion Mass | Dwell Time(s) | Collision Energy |
|---|---|---|---|---|
| Methocarbamol | 242.1 | 118 | 0.20 | 11 |
| Methocarbamol | 242.1 | 199* | 0.20 | 8 |
| Methocarbamol | 242.1 | 163 | 0.20 | 13 |
| Methocarbamol-$d_4$ | 246.1 | 203* | 0.20 | 8 |

| Analyte | Precursor Mass | Product Ion Mass | Dwell Time(s) | Collision Energy |
|---|---|---|---|---|
| Guaifenesin | 199.0 | 109 | 0.20 | 24 |
| Guaifenesin | 199.0 | 151 | 0.20 | 27 |
| Guaifenesin | 199.0 | 163* | 0.20 | 11 |
| Guaifenesin-$d_3$ | 202.0 | 166* | 0.20 | 8 |

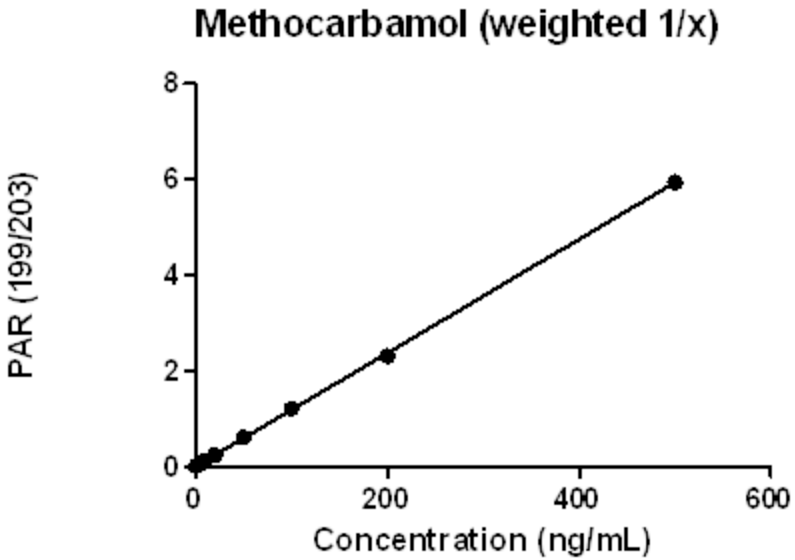
# Ion chromatograms

Total ion chromatogram (upper trace) and ion chromatograms for quantifier ion (*m/z* 199) and qualifier ions (*m/z* 163 and 118) for methocarbamol and quantifier ion for methocarbamol-$d_4$ (*m/z* 203) (bottom trace). The ion chromatograms were obtained at a methocarbamol plasma concentration of 0.5 ng/mL.



# Methocarbamol Determination

Calibration curve for determination of methocarbamol from horse plasma by LC-MS (Thermo Scientific Accela™ liquid chromatograph and Quantum Ultra triple stage quadrupole mass spectrometer with HESI) using methocarbamol-$d_4$ as internal standard. The calibration curve ranged from 0.5 ng/mL to 500 ng/mL of plasma and was weighted by 1/x where x is the nominal concentration of methocarbamol.



# Calibration Data

Table 1. Linear regression analysis data for four sets of calibration data acquired during method validation studies.

| **Methocarbamol in Horse Plasma** | | | | |
|---|---|---|---|---|
| **Calibration Curve Parameters: Immediate Analysis** | | | | |
| | | | | |
| **Range: 0.1-500 ng/mL of plasma** | | | | |
| **Weighted (1/x) linear fit** | | | | |
| | | | | |
| **Run** | **Date** | **Y-intercept** | **Slope** | **$R^2$** |
| Set 1 | 7/15/2010 | 0.003299 | 0.01187 | 0.9993 |
| Set 2 | 7/16/2010 | 0.001366 | 0.01212 | 0.9993 |
| Set 3 | 7/17/2010 | 0.002806 | 0.01374 | 0.9997 |
| Set 4 | 7/20/2010 | 0.001243 | 0.01326 | 0.9999 |
| *AVG* | | 0.002179 | 0.012748 | |
| *St. Dev.* | | 0.00103 | 0.000897 | |

# Control Samples

Table 2. Individual results for the determination of methocarbamol in control samples analyzed during validation studies are shown to the right. Four batches of positive control samples were prepared and analyzed over four days. Five replicate samples at each of five concentrations were prepared and analyzed for each batch analysis.

| Sample | Replicate | Nominal Concentration | | | | |
|---|---|---|---|---|---|---|
| | | QC1 1.0 (ng/mL) | QC2 25 (ng/mL) | QC3 75 (ng/mL) | QC4 250 (ng/mL) | QC5 400 (ng/mL) |
| **Batch 1** | **1** | 1.03 | 26.5 | 77.4 | 228.9 | 392.4 |
| | **2** | 1.02 | 26.1 | 76.4 | 254.5 | 405.7 |
| | **3** | 1.00 | 24.7 | 79.1 | 247.5 | 410.7 |
| | **4** | 0.98 | 26.5 | 74.3 | 248.3 | 387.1 |
| | **5** | 1.01 | 25.3 | 72.6 | 241.3 | 415.8 |
| **Batch 2** | **1** | 0.96 | 28.5 | 78.8 | 231.5 | 416.6 |
| | **2** | 0.99 | 26.2 | 76.6 | 261.6 | 382.0 |
| | **3** | 1.04 | 27.0 | 80.6 | 249.4 | 405.8 |
| | **4** | 0.98 | 26.9 | 79.2 | 242.8 | 395.1 |
| | **5** | 1.11 | 27.3 | 84.0 | 250.6 | 402.0 |
| **Batch 3** | **1** | 0.99 | 27.3 | 77.4 | 258.9 | 408.2 |
| | **2** | 1.08 | 25.9 | 77.6 | 251.5 | 397.3 |
| | **3** | 0.95 | 27.9 | 80.6 | 252.6 | 400.9 |
| | **4** | 0.86 | 26.6 | 79.2 | 252.3 | 402.6 |
| | **5** | 1.09 | 26.5 | 84.0 | 255.8 | 401.4 |
| **Batch 4** | **1** | 1.07 | 24.4 | 72.2 | 259.5 | 401.9 |
| | **2** | 0.99 | 24.3 | 75.3 | 236.3 | 403.1 |
| | **3** | 0.99 | 23.8 | 72.5 | 254.7 | 407.7 |
| | **4** | 0.92 | 27.1 | 75.3 | 231.7 | 386.7 |
| | **5** | 1.06 | 27.7 | 78.0 | 252.3 | 395.8 |

# Intra- and Inter-batch statistics

Table 3. Intra-batch and inter-batch summary statistics for the determination of methocarbamol in control samples analyzed during validation studies are shown to the right. Four batches of positive control samples were prepared over four days. Five replicate samples at each of five concentrations were prepared and analyzed for each batch analysis.

| Sample | Intra-batch (within-run) Statistics | | | | | | |
|---|---|---|---|---|---|---|---|
| | n | Mean | SD | % RSD | %RE | Ancillary | Statistics |
| *QC1* | 5 | 1.005 | 0.021 | 2.11 | 0.53 | **MSw =** | 0.004 |
| **1.0 ng/mL** | 5 | 1.016 | 0.060 | 6.00 | 1.57 | **MSb =** | 0.000 |
| | 5 | 0.996 | 0.094 | 9.44 | -0.38 | **MSt =** | 0.004 |
| | 5 | 1.006 | 0.061 | 6.05 | 0.58 | **st =** | 0.060 |
| | | | | | | **sb =** | 0.000 |
| | | | | | | **p =** | 4 |
| **Intra-batch statistics (Pooled):** | 5 | 1.006 | 0.060 | 6.0 | 0.6 | | |
| **Inter-batch statistics (ANOVA):** | 20 | 1.006 | 0.060 | 6.0 | 0.6 | | |
| *QC2* | 5 | 25.8 | 0.796 | 3.18 | 3.29 | **MSw =** | 1.264 |
| **25 ng/mL** | 5 | 27.2 | 0.833 | 3.33 | 8.67 | **MSb =** | 3.331 |
| | 5 | 26.9 | 0.783 | 3.13 | 7.40 | **MSt =** | 1.590 |
| | 5 | 25.5 | 1.77 | 7.06 | 1.81 | **st =** | 1.261 |
| | | | | | | **sb =** | 0.643 |
| | | | | | | **p =** | 4 |
| **Intra-batch statistics (Pooled):** | 5 | 26.3 | 1.12 | 4.50 | 5.29 | | |
| **Inter-batch statistics (ANOVA):** | 20 | 26.3 | 1.30 | 5.18 | 5.29 | | |
| *QC3* | 5 | 76.0 | 2.53 | 3.37 | 1.29 | **MSw =** | 6.061 |
| **75 ng/mL** | 5 | 79.8 | 2.74 | 3.65 | 6.46 | **MSb =** | 26.445 |
| | 5 | 75.5 | 2.16 | 2.88 | 0.72 | **MSt =** | 9.280 |
| | 5 | 74.6 | 2.39 | 3.18 | -0.48 | **st =** | 3.046 |
| | | | | | | **sb =** | 2.091 |
| | | | | | | **p =** | 4 |
| **Intra-batch statistics (Pooled):** | 5 | 76.5 | 2.46 | 3.28 | 2.00 | | |
| **Inter-batch statistics (ANOVA):** | 20 | 76.5 | 3.18 | 4.25 | 2.00 | | |
| *QC4* | 5 | 2441 | 9.72 | 3.89 | -2.36 | **MSw =** | 93.248 |
| **250 ng/mL** | 5 | 247.2 | 11.03 | 4.41 | -1.13 | **MSb =** | 92.746 |
| | 5 | 254.2 | 3.08 | 1.23 | 1.68 | **MSt =** | 93.169 |
| | 5 | 246.9 | 12.14 | 4.86 | -1.24 | **st =** | 9.652 |
| | | | | | | **sb =** | 0.000 |
| | | | | | | **p =** | 4 |
| **Intra-batch statistics (Pooled):** | 5 | 248.1 | 9.652 | 3.86 | -0.76 | | |
| **Inter-batch statistics (ANOVA):** | 20 | 248.1 | 9.652 | 3.86 | -0.76 | | |
| *QC5* | 5 | 402.3 | 12.17 | 3.04 | 0.59 | **MSw =** | 98.656 |
| **400 ng/mL** | 5 | 400.3 | 12.83 | 3.21 | 0.07 | **MSb =** | 12.085 |
| | 5 | 402.1 | 3.95 | 0.99 | 0.52 | **MSt =** | 84.910 |
| | 5 | 399.0 | 8.11 | 2.03 | -0.24 | **st =** | 9.215 |
| | | | | | | **sb =** | 0.000 |
| | | | | | | **p =** | 4 |
| **Intra-batch statistics (Pooled):** | 5 | 400.9 | 9.215 | 2.30 | 0.23 | | |
| **Inter-batch statistics (ANOVA):** | 20 | 400.9 | 9.215 | 2.30 | 0.23 | | |

# Accuracy and Precision

Table 4. Summary statistics for the determination of methocarbamol in control samples analyzed during validation studies are shown to the right. Five batches of positive control samples were prepared. Four replicate samples at each of five concentrations were prepared and analyzed for each batch analysis. Statistical analysis was performed according to DeSilva *et al*., *Pharmaceutical Research* **20**(11): 1885-1900, 2003.

| Characteristic | Statistic | Nominal Concentration | | | | |
|---|---|---|---|---|---|---|
| | | 1.0 (ng/mL) | 25 (ng/mL) | 75 (ng/mL) | 250 (ng/mL) | 400 (ng/mL) |
| # of Results | N | 20 | 20 | 20 | 20 | 20 |
| Accuracy | Mean Bias (%RE) | 0.6 | 5.3 | 2.0 | -0.8 | 0.2 |
| | LCL | -0.692 | 0.097 | -2.879 | -3.503 | -0.385 |
| | UCL | 1.840 | 10.487 | 6.879 | 1.979 | 0.852 |
| Precision | Intra-batch (%CV) | 6.0 | 4.5 | 3.3 | 3.9 | 23.3 |
| | Inter-batch (%CV) | 6.0 | 5.2 | 4.2 | 3.9 | 2.3 |
| Accuracy + Precision | \|Mean\| + Inter-batch | 6.532 | 10.472 | 6.245 | 4.623 | 2.537 |
| 90% Expectation | Lower Limit (%RE) | -9.8 | -4.3 | -6.2 | -7.6 | -3.8 |
| Tolerance Interval | Upper Limit (%RE) | 10.9 | 14.9 | 10.2 | 6.1 | 4.2 |

# Matrix Effects

Table 5. Matrix effect, Extraction Efficiency, and Process Efficiency data for methocarbamol in horse plasma determined by the methods described by Matuszewski *et al*., *Anal. Chem.* **75**: 3019-3030 (2003).

| Methocarbamol Concentration, ng/mL | Absolute Matrix Effect, % | Extraction Efficiency (%) | Process Efficiency (% |
|---|---|---|---|
| 1 | 92.00% | 78.85% | 72.54% |
| 25 | 104.31% | 51.29% | 53.50% |
| 75 | 99.97% | 60.16% | 60.14% |
| 250 | 124.30% | 63.87% | 79.39% |
| 400 | 124.38% | 61.59% | 76.60% |

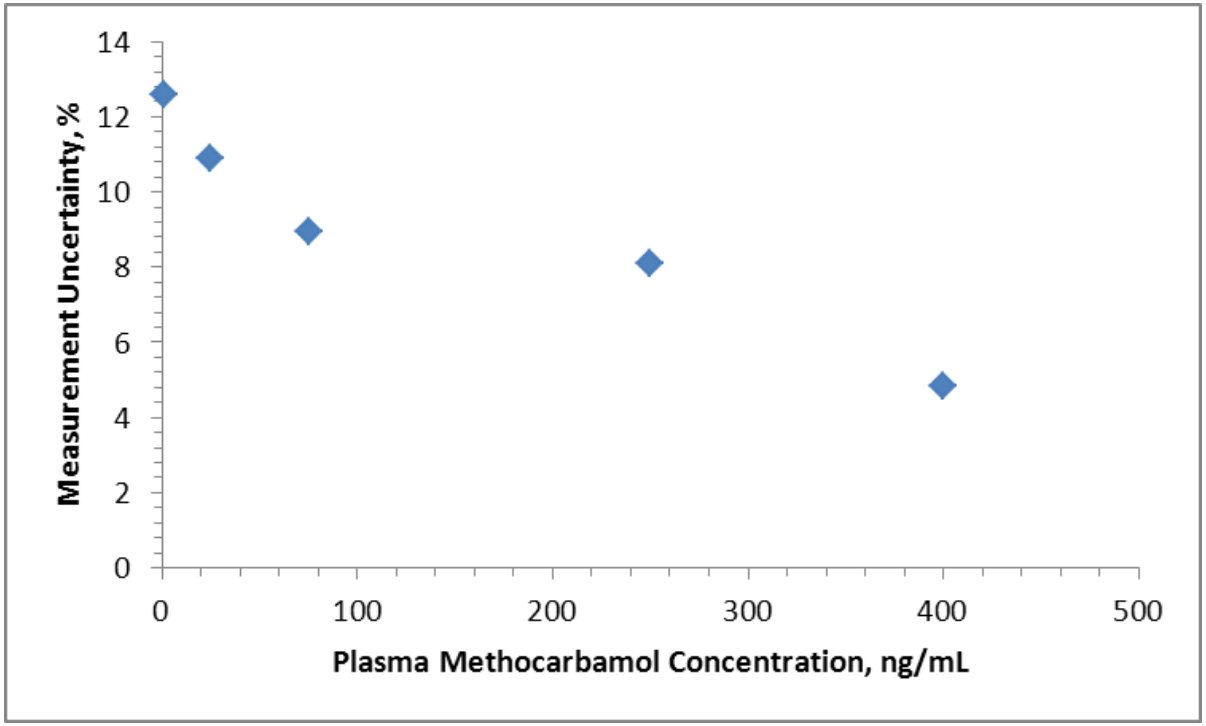
# Measurement Uncertainty

Table 6. Measurement uncertainty for methocarbamol in control samples by liquid chromatography-mass spectrometry based on data obtained during the validation of the method by Marc Rumpler and Richard Sams at the Florida Racing Laboratory, University of Florida (July 2010).

| Concentration, ng/mL | n | $S_R$, ng/mL | RSD, % | k | U, % |
|---|---|---|---|---|---|
| 1 | 20 | 0.06 | 6.0 | 2.1 | 12.6 |
| 25 | 20 | 1.3 | 5.18 | 2.1 | 10.9 |
| 75 | 20 | 3.18 | 4.25 | 2.1 | 8.92 |
| 250 | 20 | 9.652 | 3.86 | 2.1 | 8.11 |
| 400 | 20 | 9.215 | 2.3 | 2.1 | 4.83 |

# Measurement Uncertainty

Plot of percent measurement uncertainty (U) as a function of the plasma concentration of methocarbamol. Calculations were based on twenty determinations of each control sample and a 95% confidence level (University of Florida, July 2010).



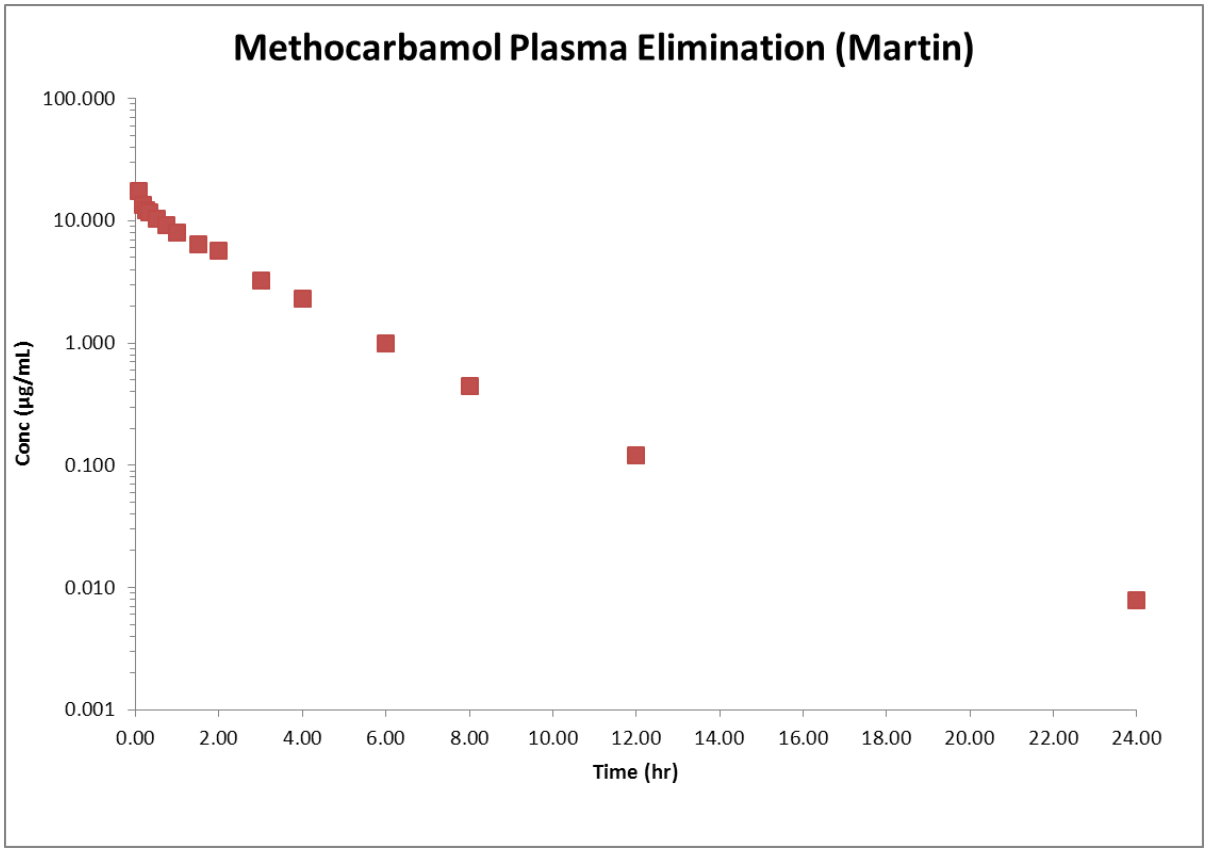
# Plasma concentrations from PK study

Plasma methocarbamol concentrations from the six horse IV bolus dose study as determined by Marc Rumpler at the UF Racing Laboratory using the validated procedure for determining methocarbamol in horse plasma.

| Plasma Methocarbamol Concentration (microgram/milliliter) | | | | | | |
|---|---|---|---|---|---|---|
| Time (min) | Bob | Martin | True | Rupert | Slip | Des |
| 0.08 | 33.61 | 21.1 | 26.34 | 20.86 | 16.92 | 20.34 |
| 0.17 | 17.22 | 16.03 | 18.2 | 14.95 | 15.00 | 13.33 |
| 0.25 | 13.83 | 14.62 | 13.5 | 12.98 | 12.93 | 13.01 |
| 0.33 | 11.99 | 14.14 | 11.43 | 11.65 | 10.19 | 11.56 |
| 0.50 | 10 | 12.36 | 10.6 | 11.19 | 9.96 | 11.03 |
| 0.75 | 8 | 11.16 | 8.15 | 8.71 | 8.93 | 10.66 |
| 1.00 | 5.53 | 9.61 | 8.13 | 7.27 | 6.81 | 8.77 |
| 1.50 | 4.63 | 7.68 | 6.11 | 6.22 | 5.25 | 6.34 |
| 2.00 | 3.32 | 6.89 | 4.94 | 4.42 | 3.87 | 5.81 |
| 3.00 | 1.72 | 3.93 | 2.74 | 2.16 | 1.84 | 3.61 |
| 4.00 | 0.97 | 2.79 | 1.98 | 1.2 | 1.28 | 2.64 |
| 6.00 | 0.408 | 1.18 | 0.84 | 0.595 | 0.567 | 1.26 |
| 8.00 | 0.121 | 0.535 | 0.312 | 0.239 | 0.206 | 0.528 |
| 12.00 | 0.0285 | 0.146 | 0.0793 | 0.0533 | 0.0564 | 0.15 |
| 24.00 | 0.00105 | 0.00947 | 0.00332 | 0.00202 | 0.00188 | 0.00791 |

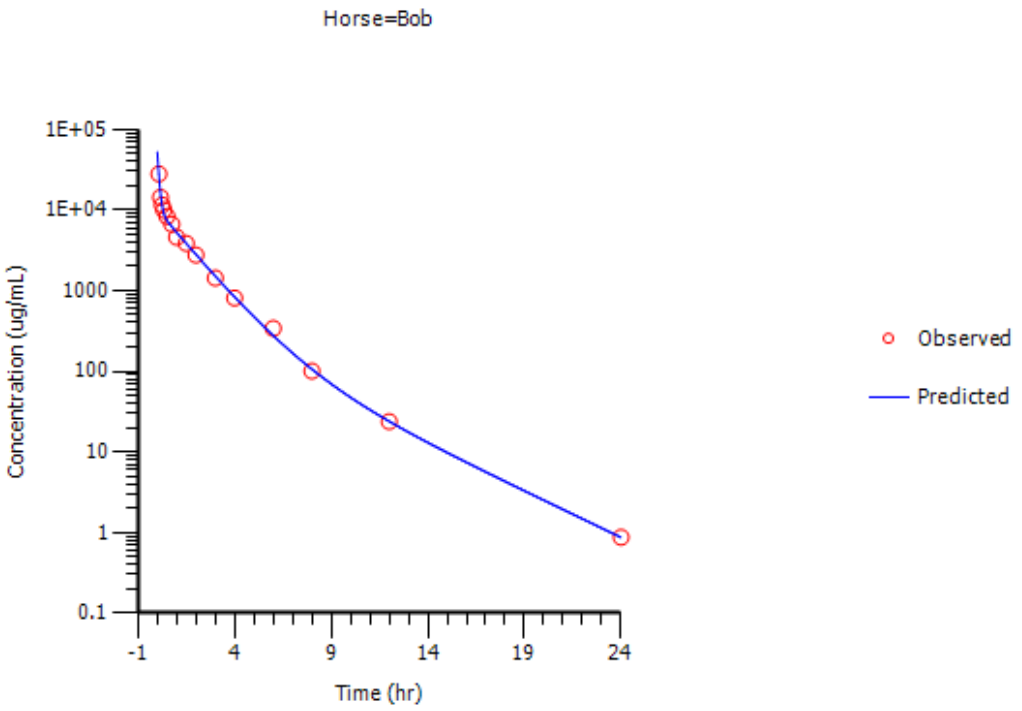
# Sample Analyses

Plot of plasma methocarbamol concentrations in a Thoroughbred horse (Martin) after single dose intravenous administration of 15 mg/kg body weight. Plasma concentrations were determined by a validated LC-MS method characterized by a lower limit of quantitation of 0.5 ng/mL. Samples containing methocarbamol at concentrations greater than 500 ng/mL were diluted into the range of the calibration curve (0.5-500 ng/mL). No guaifenesin was detected in any post-administration samples.



# Pharmacokinetic Analysis

Plasma concentration versus time data were analyzed by non-linear least squares regression analysis using Phoenix® WinNonlin™ software from Pharsight (St. Louis, MO). Data were weighted by 1/y2 where y is the measured plasma concentration. A three compartment model provided the best fit of the model to the data for each horse based on AIC and goodness of fit criteria.



# Methocarbamol – IV PK Parameters

- Secondary parameters obtained from the pharmacokinetic analysis of plasma concentration versus time data for each horse (n=6) after IV administration.
- Values for the total clearance are about half of hepatic blood flow suggesting that the extent of hepatic metabolism is large and that a high first-pass effect after oral administration should be expected.

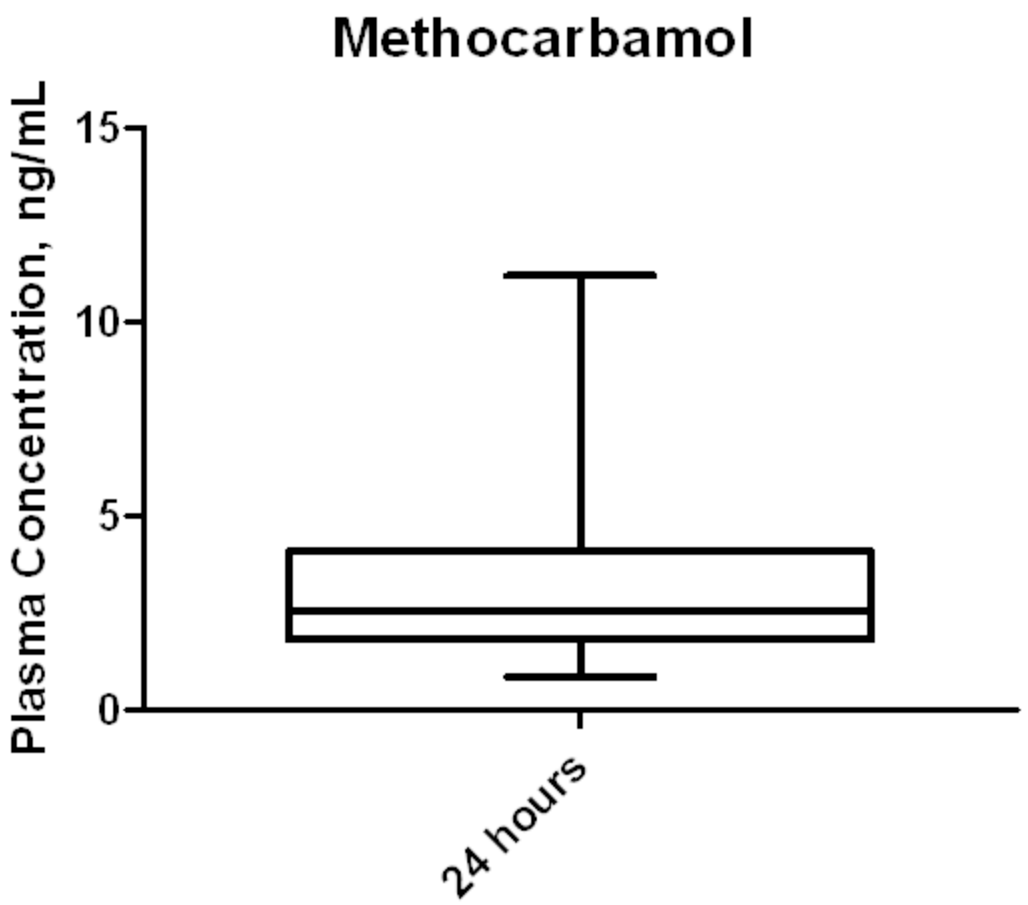
| Methocarbamol pharmacokinetics after intravenous administration | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Pharmacokinetic parameters for methocarbamol | | | | | | | | | |
| Horse Name | half-life | Vss | Vss | CL | CL | AUC | AUMC | MRT | Fitting of data |
| | hr | L | L/kg | L/hr | mL/min/kg | hr*ug/mL | $hr^2$ug/mL | hr | AIC |
| Bob | 2.63 | 483 | 0.943 | 333 | 10.83 | 23.93136 | 34.74352 | 1.45 | -88.735 |
| Des | 3.29 | 585 | 1.081 | 233 | 7.19 | 34.78335 | 87.17088 | 2.51 | -141.187 |
| Martin | 3.64 | 524 | 0.987 | 213 | 6.68 | 37.40933 | 92.01295 | 2.46 | -151.575 |
| Rupert | 2.52 | 564 | 1.064 | 307 | 9.64 | 25.92271 | 47.69392 | 1.84 | -171.627 |
| Slip | 2.46 | 612 | 1.212 | 323 | 10.67 | 23.43593 | 44.38726 | 1.89 | -173.699 |
| True | 4.71 | 557 | 1.030 | 270 | 8.33 | 30.00451 | 61.82655 | 2.06 | -59.2235 |

# Distribution of plasma methocarbamol

This graph illustrates the distribution of plasma methocarbamol concentrations in samples collected from Thoroughbred research horses 24 hours after IV administration of a single dose of 15 mg/kg of body weight to each horse.

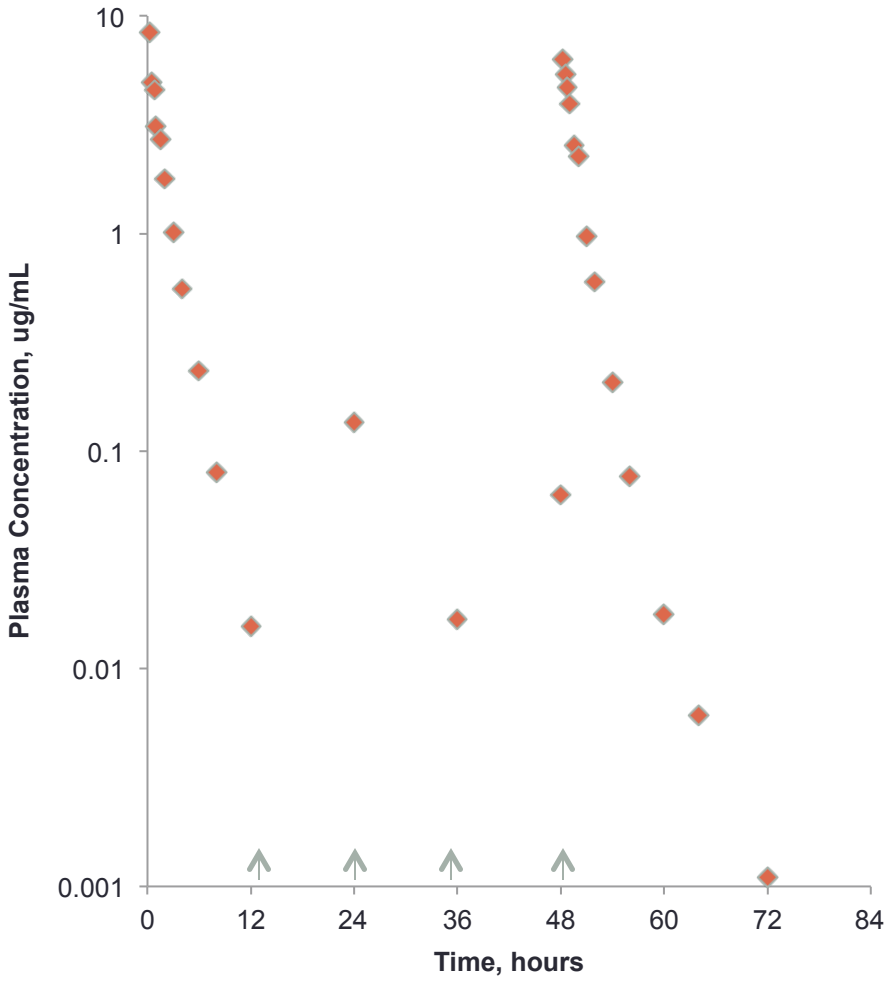
Plasma concentrations were determined by a validated LC-MS method using a stable isotope labeled internal standard to improve accuracy and precision. The method was characterized by a lower limit of quantification of 0.5 ng/mL.

Values ranged from 1.04 -13.4 ng/mL. The 99/95 tolerance limit was 32 ng/mL at 24 hours.



# Sample Analyses

Plot of plasma methocarbamol concentrations in a Thoroughbred horse (Bob) after multiple dose oral administration of 5000 mg q 12 h. Plasma concentrations were determined by a validated LC-MS method as described previously. Samples containing methocarbamol at concentrations greater than 500 ng/mL were diluted into the range of the calibration curve (0.5-500 ng/mL). Guaifenesin was detected in post-administration samples.

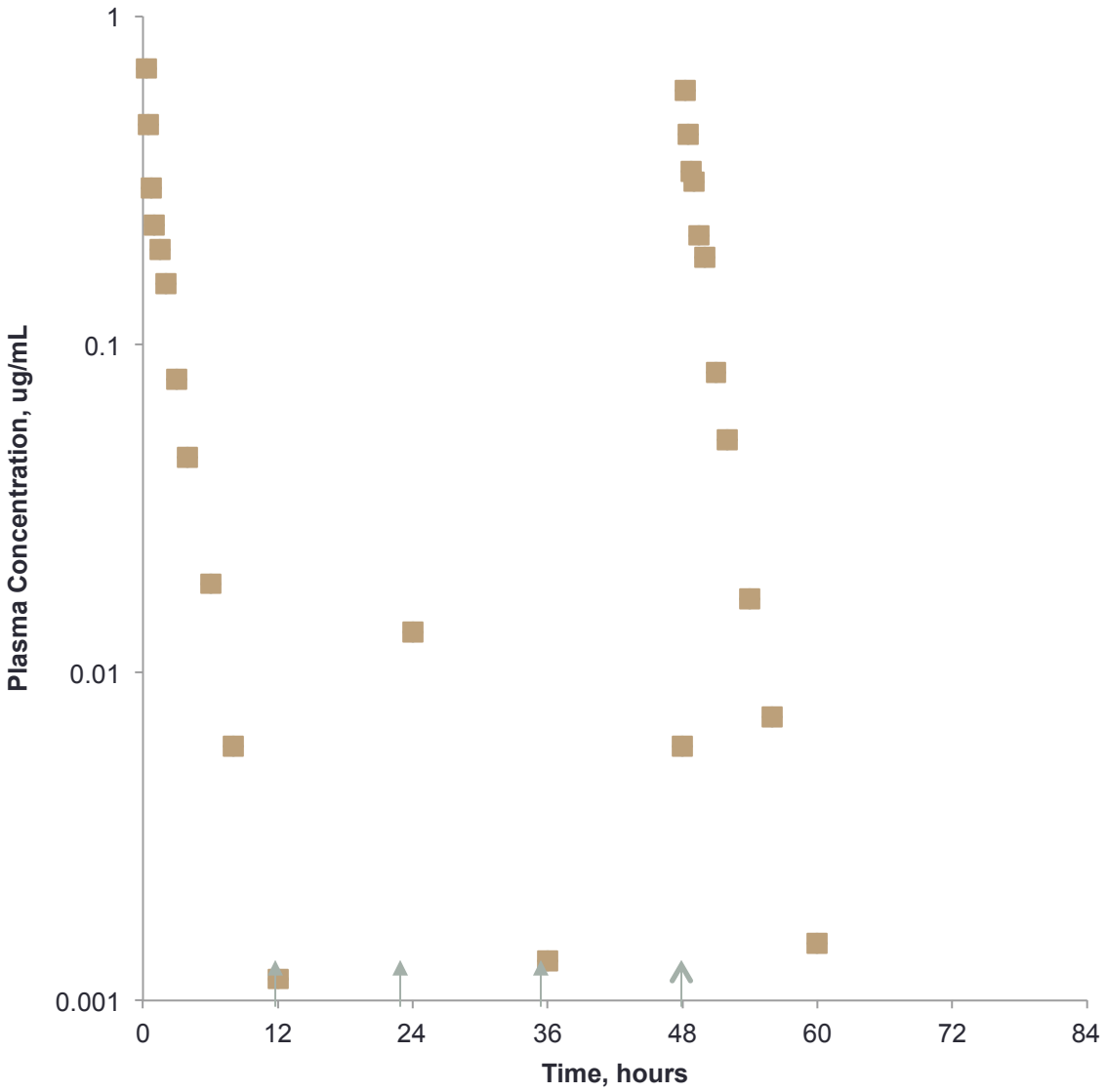


# Sample Analyses

Plot of plasma guaifenesin concentrations in a Thoroughbred horse (Bob) after multiple dose oral administration of 5000 mg q 12 h.

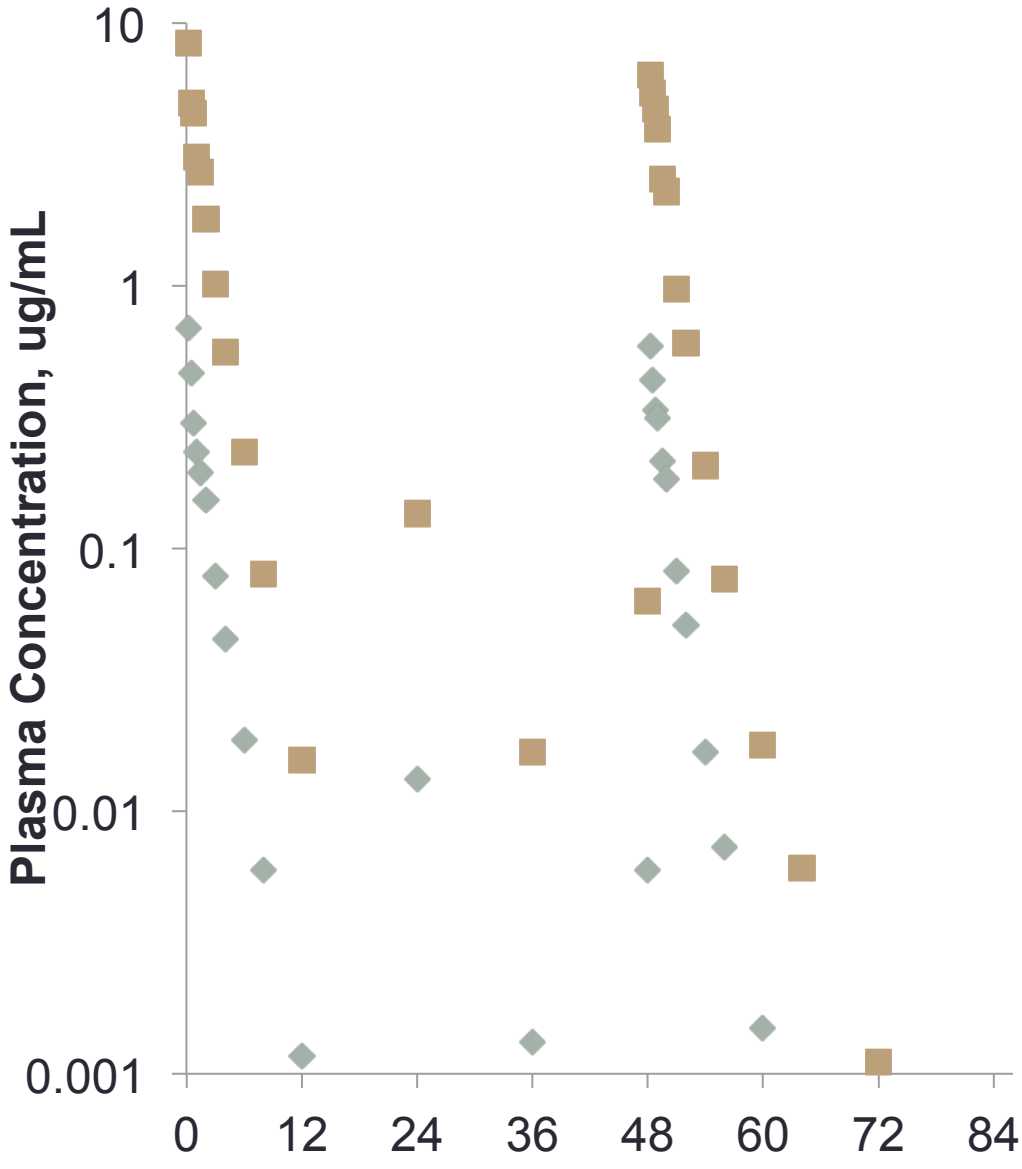
Plasma concentrations were determined by a validated LC-MS method as described previously.

Guaifenesin was detected in post-administration samples. Guaifenesin concentrations were approximately 10% of the corresponding methocarbamol concentration.



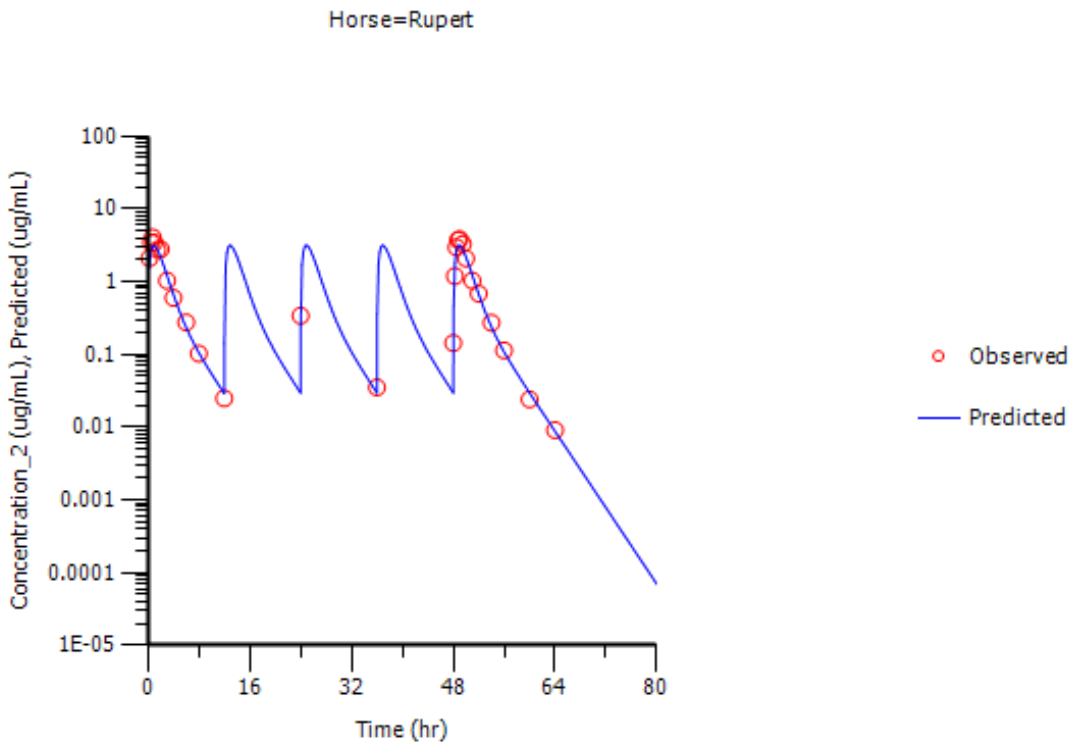
# Sample Analyses

Plot of both methocarbamol and guaifenesin plasma concentrations after multiple dose oral administration to a Thoroughbred horse (Bob) illustrating the dependence of guaifenesin on methocarbamol concentrations.



# Pharmacokinetic Analysis

Plasma concentration versus time data after multiple dose oral administration were analyzed by non-linear least squares regression analysis using Phoenix® WinNonlin™ software from Pharsight (St. Louis, MO). Data were weighted by $1/y^2$ where y is the measured plasma concentration. A two compartment model with first order absorption provided the best fit of the model to the data for each horse based on AIC and goodness of fit criteria.



# Methocarbamol – Oral PK

- Pharmacokinetic parameter estimates for methocarbamol after oral administration to horses.

| Pharmacokinetic Parameter Methocarbamol | Name of Horse | | | | | |
|---|---|---|---|---|---|---|
| | Bob | Des | Martin | Rupert | Slip | True |
| Elimination rate constant ($hrs^{-1}$) | 0.279 | 0.142 | 0.199 | 0.313 | 0.210 | 0.282 |
| Half-life (hrs) | 2.48 | 4.88 | 3.47 | 2.21 | 3.30 | 2.45 |
| Oral Availability F | 0.728 | 0.557 | 0.432 | 0.555 | 0.533 | 0.448 |
| Cl/F (L/hr) | 440.5 | 423.2 | 493.0 | 553.0 | 606.0 | 603.1 |
| Cl/F (L/hr/kg) | 0.860 | 0.782 | 0.929 | 1.043 | 1.200 | 1.115 |
| $AUC_{first\ dose}$ (hr*ug/mL) | 11.36799 | 12.3508 | 12.2027 | 9.7787 | 8.3882 | 10.0352 |
| $AUC_{last\ dose}$ (hr*ug/mL) | 12.6371 | 13.2714 | 24.3104 | 13.0904 | 23.2207 | 13.2691 |
| $AUC_{last}/AUC_{first}$ | 1.11 | 1.07 | 1.99 | 1.34 | 2.77 | 1.32 |
| $AUMC_{last}$ ($hr^2$*ug/mL) | 20.026 | 33.613 | 38.761 | 21.969 | 24.448 | 23.709 |
| $MRT_{oral}$ (hrs) | 1.80 | 2.87 | 2.84 | 2.28 | 2.66 | 2.68 |
| MRT (IV) (hrs) | 1.45 | 2.51 | 2.46 | 1.84 | 1.89 | 2.06 |
| MAT oral (hrs) | 0.35 | 0.36 | 0.38 | 0.44 | 0.77 | 0.62 |
| Absorption rate constant ($hrs^{-1}$) | 2.87 | 2.77 | 2.65 | 2.28 | 1.31 | 1.62 |
| AIC fitting of data | -254 | -218 | -203 | -247 | -195 | -188 |

# Methocarbamol – Oral PK

- Pharmacokinetic parameter estimates for guaifenesin after oral methocarbamol administration.

| | Guaifenesin Pharmacokinetic data in horses administered methocarbamol | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Pharmacokinetic parameters in horses | | | | | | | | |
| | Half-life | $AUC_{m,last}$/ $AUC_{m,first}$ | $AUC_{0\text{-}12\ hr}$ | $AUC_{last}$ | $AUC_m/AUC_p$ | $AUC_m$/ $AUC_p$ | AUMC | MRT | AIC |
| **Horse Name** | hr | | hr*ug/mL | hr*ug/mL | First dose | Last dose | $hr^2$*ug/mL | hr | |
| **Bob** | 1.47 | 1.195 | 0.891 | 1.07 | 0.078 | 0.0842 | 1.6762 | 1.82 | 0.966223 |
| **Des** | 1.42 | 1.208 | 1.36 | 1.65 | 0.110 | 0.124 | 4.0723 | 2.81 | 0.74121 |
| **Martin** | 2.10 | 2.179 | 0.851 | 1.85 | 0.0698 | 0.0763 | 2.8647 | 2.74 | 0.318158 |
| **Rupert** | 0.941 | 1.492 | 0.631 | 0.942 | 0.0645 | 0.0719 | 1.6286 | 2.47 | 0.177344 |
| **Slip** | 2.72 | 2.405 | 0.912 | 2.19 | 0.109 | 0.0944 | 2.3449 | 2.52 | 0.344581 |
| **True** | 2.62 | 1.295 | 1.16 | 1.50 | 0.115 | 0.113 | 2.5654 | 2.81 | 0.665762 |

# Pharmacokinetic Analysis

- Summary of pharmacokinetic parameter estimates for methocarbamol after intravenous and multiple dose oral administration to each of six horses in a cross-over study.
- Half-lives after IV and oral administration are not different.
- The absorption rate constant is faster than the elimination rate constant.
- Accumulation is not significant on multiple dosing.

| Intravenous | | | | |
|---|---|---|---|---|
| **Parameter** | **Units** | **Median** | **Minimum** | **Maximum** |
| *Half-life* | h | 2.96 | 2.46 | 4.71 |
| *Vss* | L/kg | 1.05 | 0.943 | 1.21 |
| *Cl* | ml/min/kg | 8.99 | 6.68 | 10.83 |
| *AUC* | h*ug/mL | 28.0 | 23.4 | 37.4 |
| *AUMC* | $h^2$*ug/mL | 54.8 | 34.7 | 92.0 |
| *MRT* | h | 1.98 | 1.45 | 2.51 |
| Oral | | | | |
| *k elimination* | $h^{-1}$ | 0.245 | 0.142 | 0.313 |
| *Half-life* | h | 2.89 | 2.21 | 4.88 |
| *Oral Availability* | F | 0.544 | 0.432 | 0.728 |
| *Cl/F* | L/hr | 523 | 423 | 606 |
| *Cl/F* | L/hr/kg | 0.986 | 0.782 | 1.20 |
| *AUCfirst dose* | hr*ug/mL | 10.7 | 8.39 | 12.4 |
| *AUClast dose (hr*ug/mL)* | | 13.3 | 12.6 | 24.3 |
| *AUClast/AUCfirst* | | 1.33 | 1.07 | 2.77 |
| *AUMClast* | $hr^2$*ug/mL | 24.1 | 20.0 | 38.8 |
| *MRToral* | h | 2.67 | 1.8 | 2.87 |
| *MAT oral* | h | 0.410 | 0.350 | 0.770 |
| *k absorption* | $h^{-1}$ | 2.47 | 1.31 | 2.87 |

# Methocarbamol Summary

- IV Study
  - Plasma methocarbamol concentrations were determined by a validated UPLC-LCMS method characterized by a method limit of detection of 0.1 ng/mL and a lower limit of quantitation of 0.5 ng/mL.
  - The method was validated with regard to accuracy, precision, specificity, sensitivity, linearity, analyte stability, ruggedness, and transferability.
  - Plasma methocarbamol concentrations were determined for 24 hours after intravenous administration of a single dose (15 mg/kg of body weight) of methocarbamol to each of twenty Thoroughbred horses.
  - Plasma methocarbamol concentrations at 24 hours after dosing (n=20) were analyzed and a 99/95 upper tolerance limit of 32 ng/mL was calculated.
  - Plasma concentration versus time data in six horses were subjected to PK analysis and a three compartment open model was selected based on AIC and goodness of fit criteria.
  - Guaifenesin was not detected in any sample collected after intravenous methocarbamol administration.

# Methocarbamol Summary

- Multiple Dose Oral Study
  - Plasma methocarbamol and guaifenesin concentrations were determined periodically after repeat dose oral administration of methocarbamol tablets to each of six Thoroughbred horses.
  - The horses were administered ten 500 mg tablets of methocarbamol orally every 12 hours to each of the six horses in the IV dose group.
  - Plasma concentration versus time data in these horses were subjected to PK analysis and a two compartment open model with first order absorption was selected based on AIC and goodness of fit criteria.
  - Methocarbamol was rapidly absorbed after oral administration. Minimal accumulation occurred on repeat dose administration.
  - Systemic bioavailability after oral administration was incomplete and was attributed to first pass hepatic metabolism.
  - Guaifenesin was detected in most samples collected after oral methocarbamol administration; guaifenesin concentrations were about 10% of the corresponding methocarbamol concentration.

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