Title: The intravenous pharmacokinetics of butorphanol and detomidine dosed in combination compared with individual dose administrations to exercised horses

Short Running Title: Pharmacokinetics of butorphanol and detomidine in horse

Authors: Stuart W. Paine[1], Jane Bright[2], James P. Scarth[2], Pamela R. Hincks[2], Clive M. Pearce[2], Colette Hannan[3], Marc Machnik[4], Lynn Hillyer[5,6]

[1]School of Veterinary Medicine and Science, University of Nottingham, College Road, Sutton Bonington, Leicestershire, LE12 5RD, UK
[2] LGC Ltd, Newmarket Road, Fordham, Cambridgeshire, CB7 5WW, UK
[3] BHP Racing Laboratory, New Road, Thomondgate, Limerick, Ireland
[4] Institute of Biochemistry, German Sport University, D50933 Cologne, Germany
[5] British Horse Racing Authority, 75 High Hoborn, London, WC1V 6LS, UK
[6]Current address: Irish Horseracing Regulatory Board, The Curragh, Kildare, Co Kildare, Ireland

KEYWORDS (6) Butorphanol, detection time, detomidine, drug-drug interactions, horse, pharmacokinetics.

Abstract

In equine and racing practice, detomidine and butorphanol are commonly used in combination for their sedative properties. The aim of the study was to produce Detection Times to better inform European veterinary surgeons, so that both drugs can be used appropriately under regulatory rules. Three independent groups of 7, 8 and 6 horses, respectively, were given either a single intravenous administration of butorphanol (100 μg/kg), a single intravenous administration of detomidine (10 μg/kg) or a combination of both at 25 (butorphanol) and 10 (detomidine) μg/kg. Plasma and urine concentrations of butorphanol, detomidine and 3-hydroxydetomidine at predetermined time points were measured by liquid chromatography-tandem mass spectrometry (LC-MS/MS). The intravenous pharmacokinetics of butophanol dosed individually compared with co-administration with detomidine had approximately a two-fold larger clearance (646 ± 137 versus 380 ± 86 ml/hr/kg) but similar terminal half-life (5.21 ± 1.56 versus 5.43 ± 0.44 hr). Pseudo-steady-state urine to plasma butorphanol concentration ratios were 730 and 560, respectively. The intravenous pharmacokinetics of detomidine dosed as a single administration compared with co-administration with butorphanol had similar clearance (3278

±1412 versus 2519 ± 630 ml/hr/kg) but a slightly shorter terminal half-life (0.57 ± 0.06 versus 0.70 ± 0.11 hr). Pseudo-steady-state urine to plasma detomidine concentration ratios are 4 and 8, respectively. The 3-hydroxy metabolite of detomidine was detected for at least 35 hours in urine from both the single and co-administrations. Detection Times of 72 and 48 hours are recommended for the control of butorphanol and detomidine, respectively, in horseracing and equestrian competitions.

Introduction

The combination of detomidine and butorphanol is frequently used in clinical equine practice to provide a degree of systemic analgesia alongside sedation for minor procedures, such as wound repair or dental work. Whilst they may also be used singly, the combination affords better predictability and efficacy with lower doses of butorphanol and potentiation of the sedative effect of detomidine alone - all in the interests of horse, veterinary surgeon and owner. The use of agents, such as α_2 agonist sedatives and opioid analgesics, is restricted under the rules of international horseracing in order that horses race free from the effects of medication. However, use of therapeutic medications to treat racehorses out of competition is legitimate. Therefore, to deal with the race-day scenario where a horse competes having received medication in its training programme, the term “free from the effects of medication” has to be defined. One such definition can be a drug concentration in blood plasma that is less than the concentration required for a significant therapeutic effect. Toutain & Lassourd, 2002 proposed an approach based upon the above definition that also takes into account variation in both pharmacokinetic (PK) and pharmacodynamic (PD) parameters for a population of horses. An estimate of the irrelevant plasma concentration (IPC) is based upon a pharmacologically effective plasma drug concentration divided by an appropriate safety factor. Also, with knowledge of urine PK parameters an irrelevant urine concentration (IUC) can also be estimated. The IPC and IUC can be used for the purpose of deriving drug screening limits, which in turn, can be used in conjunction with drug elimination profiles to obtain appropriate detection times (DT) for the matrices concerned. The DT is the time post last therapeutic administration when the plasma and urine drug concentrations for all horses in the study drop below the IPC and IUC.

Detomidine is an α_2-agonist, used as a sedative primarily in horses, and is usually available as the salt detomidine hydrochloride (Elfenbein et al., 2009). Machnik et al., 2006 described an intravenous (IV) PK study of detomidine hydrochloride and its metabolites 3-hydroxydetomidine and 3-carboxydetomidine at a dose of 20 μg/kg in ten horses for the purposes of

developing a detection time. Grimsrud et al., 2009 published a two way cross over study that investigated the PK of detomidine and its metabolites from both IV and intramuscular (IM) administrations at a dose of 30 μg/kg in eight horses. Hubbell et al., 2009 reported the effect of the timing of the administration of detomidine relative to physical exertion by administering the drug IV at a dose of 40 μg/kg either at rest or one minute after exercising on a treadmill.

Butorphanol is a synthetic opioid analgesic and is used for pain relief in horses. It is administered either IM or IV and is also commonly paired with sedatives such as detomidine, to provide analgesia in addition to sedation during veterinary procedures. Hannan et al., 2004 described an IV PK study of butorphanol tartrate at therapeutic dose (100 μg/kg) in 6 horses and this was followed by Sellon et al., 2008 two-way cross-over study of the pharmacokinetics of butorphanol after IV and IM administration (80 μg/kg) in six horses. Arguedas et al., 2008 reported the PK and physiological/behavioural effects of butorphanol after IV and IM administration to six foals aged between 3 and 8 days. More recently, Knych et al., 2012 reported on the PK/PD of butorphanol following intravenous administration to the horse utilising a highly sensitive liquid chromatography mass spectrometry (LC-MS) assay.

Although the individual PK of butorphanol and detomidine have been studied extensively there is no reported PK for the intravenous PK of butorphanol and detomidine administered in combination. The purpose of the study presented herein was to determine the plasma and urine PK of detomidine and butorphanol co-administered using a highly sensitive LC-MS/MS method and compare to the PK of individual IV administrations of butorphanol and detomidine at their respective clinical doses in the horse. Furthermore, this study will give guidance on the withdrawal time of butorphanol and detomidine either dosed individually or in combination prior to competition for equestrian events and horseracing.

Materials and Methods

Horses

Seven healthy Thoroughbred geldings aged 4-10 years (mean bodyweight 504 kg) were used for the individual administered butorphanol tartrate study carried out at Ballybrown Equine Clinic, Calrina, Limerick, Ireland. Eight healthy Standardbred geldings/mares aged 6-16 years (mean bodyweight 638 kg) were used for the individual administered detomidine hydrochloride study carried out at the Institute of Biochemistry, Cologne, Germany. Six healthy Thoroughbred geldings aged 4-8 years (mean bodyweight 504 kg) were used for the co-administration of butorphanol tartrate and detomidine hydrochloride study

carried out at the British Horseracing Authority's Centre for Racehorse Studies, Newmarket, UK.

Drug administration and sampling

All administration and sampling studies were carried out with ethical approval from the country specific animal research authority. A 14 gauge catheter was placed in the left external jugular vein for sampling and the drug administered into the right jugular vein. Drug doses for the different studies were; 100 and 10 μg/kg for the individual administrations of butorphanol and detomidine respectively, and 25 and 10 μg/kg for the combination administration of butorphanol and detomidine, respectively. For the combination study detomidine hydrochloride was given 5 minutes after butorphanol tartrate administration.

Pre-dose blood (10 mL) and urine (20 mL) were collected as control samples. Post dose blood (10 mL) and urine (20 mL) were collected at decreasing frequencies as described in Supplementary Tables 1-3. Catheters were removed following collection of the 24 h post dose blood sample and the remaining samples collected by direct venepuncture. All urine samples were obtained using the free catch method. Blood was centrifuged for 10 minutes and plasma stored at -20ºC until analysis. Urine was frozen immediately and stored at -20ºC and centrifuged post thaw prior to analysis.

Sample Bioanalysis:

Samples obtained following administration of butorphanol, detomidine and butorphanol/detomidine were analysed, respectively at: BHP Racing Laboratory, Limerick, Ireland, the Institute of Biochemistry, Cologne, Germany and LGC, Fordham, UK. The analytical methods were validated to ensure suitable precision and accuracy, lower limit of quantification (LLOQ), linearity, calibration range and selectivity (see results section). Supplementary Tables 4 and 5 detail the sample preparation/extraction and instrumental conditions respectively that were used by each laboratory. In addition to concentrations of 'parent' detomidine and butorphanol in plasma and urine, concentrations of 3-hydroxydetomidine were also measured since urinary concentrations of this metabolite were more abundant than parent drug; this making it a more suitable target analyte for any subsequently applied routine drug screening procedures (Machnik et al., 2006). Detomidine is also metabolised to 3-carboxydetomidine in the horse. However, this metabolite was not chosen for quantification since its chemical properties make it potentially less suitable for inclusion in subsequent routine drug screening procedures that may differ between laboratories.

Pharmacokinetic Analysis

Pharmacokinetic analyses were conducted using non-compartmental (NCA) and non-linear mixed effects methods (NLME) with Phoenix WinNonlin 8.1 (Pharsight Corporation, Cary, NC). The 2-stage NCA approach firstly involved the estimation of clearance (CL), terminal volume of distribution (Vd_z), half-life ($T_{1/2}$), mean residence time (MRT) and steady-state volume of distribution (V_{ss}) for butorphanol and detomidine. Secondly, statistical t-tests were performed on the log transformed pharmacokinetic parameters for (i) butophanol dosed individually versus in combination with detomidine and (ii) detomidine dosed individually versus in combination with butorphanol to determine any differences. Compartmental NLME PK models were applied to the plasma concentration data including below the limit of quantification (BLQ) values. Residual error was modelled on a proportional error model. An exponential random effect model was chosen to describe inter-individual variability e.g. parameter = typical parameter * $\exp^{(eta)}$. A categorical covariate for individual versus combination administration was implemented on the model parameters in a multiplicative exponential way. The model analysis started from the basic compartmental models without the covariate. Next, the contribution of the covariate to the PK parameters was assessed by a reduction in the objective function using stepwise forward inclusion. Selection of the best model was based on the lowest value of the Akaike and Bayesian Information Criteria (AIC and BIC), chi-square p-value based on the likelihood ratio test, visual inspection of the population predicted concentration versus the observed concentrations and the resulting conditional weighted residual errors. Finally, the best model was checked for robustness using a bootstrap resampling method. The effective plasma concentration (EPC) and irrelevant plasma and urine (IPC and IUC) were estimated using the Toutain and Lassourd, 2002 methodology. Briefly, the EPC was estimated from the dose divided by the mean plasma clearance over a 24 hour period. The IPC was determined by dividing the EPC by a factor of 500 and the IUC determined by multiplying the IPC by the pseudo steady-state ratio of urine to plasma concentration (Rss). The detection time was determined by the time post dose where all horses had concentrations below the IPC and IUC. Montecarlo simulations were used to determine a 95%/95% tolerance interval for a withdrawal time.

Results

Sample Bioanalysis

Following validation, the analytical methods were deemed suitable for application to the study samples. Specifically, inter- and intra-batch precision (% coefficient of variation) and accuracy (% relative error) were within 20%

(25% at the LLOQ), linearity of dilution (% relative error and % coefficient of variation) was within 20% and no significant matrix suppression or interferences were observed. Supplementary Table 6 summarises the resulting calibration ranges, LLOQs and sample dilution ranges that were validated for each method. LLOQs (LOD) for butorphanol and detomidine in plasma and urine were 10 (2) and 100 (10) pg/ml, respectively.

Intravenous plasma and urine pharmacokinetics for butorphanol tartrate (100 μg/kg) dosed individually

The plasma and urine PK profiles for butorphanol tartrate dosed at 100 μg/kg as a single IV administration are displayed in Figures 1 and 2, respectively, as red symbols and connecting dashed lines. The plasma decay curves appear to have three phases of decline entering into the third phase at approximately 5 hours. The urine PK curves display a rapid increase followed by single phase decay. The ratio of urine to plasma concentration at pseudo steady-state (Rss) is approximately 730.

Intravenous plasma and urine pharmacokinetics for butorphanol tartrate (25 μg/kg) co-administered with 10 μg/kg detomidine hydrochloride

The plasma and urine PK profiles for butorphanol tartrate dosed at 25 μg/kg in combination with 10 μg/kg detomidine hydrochloride as an IV administration are displayed in Figures 1 and 2, respectively, as black symbols and solid connecting lines. The plasma decay curves appear to have two phases of decline entering into the second phase at approximately 3 hours. The urine PK curves display a rapid increase followed by single phase decay. The ratio of urine to plasma concentration at pseudo steady-state (Rss) is approximately 560.

Butorphanol Plasma PK Analysis

Table 1 displays the average plasma NCA PK parameters with standard deviations computed from the PK profiles and suggests that in this study butorphanol co-administered with detomidine has a statistically lower clearance ($p < 0.05$) and longer mean residence time (MRT) ($p < 0.05$) when compared to butorphanol dosed on its own at 100 μg/kg . However, there was no significant difference between terminal half-life or steady-state volume of distribution (Vss) which are approximately 5 hours and 1 L/kg in both cases, respectively.

The most parsimonious NLME model obtained was a 3 compartment model, random effects included on all parameters with partial correlation (V_1,CL_1,V_3 and CL_2,V_2) with the covariate implemented on the parameters clearance from the central compartment (CL_1) and distribution clearance to and from the third

compartment (CL_3). The influence of the covariate for individual versus combination administration on CL_1 and CL_3 are as follows:

$$CL_1 = \text{Typical Value} * \exp^{(-0.666 * (\text{administration}=1))} * \exp^{(CL_1 \text{eta})}$$

$$CL_3 = \text{Typical Value} * \exp^{(-2.16 * (\text{administration}=1))} * \exp^{(CL_3 \text{eta})}$$

Where the Typical Value (TV) is the population fixed effect value for the parameter and administration = 1 refers to the case for combination administration.

Table 2 shows the outputted PK parameters for the most parsimonious model which were encompassed by the 2.5 and 97.5% confidence intervals of the bootstrap resampling analysis. Clearance values were similar between the NCA and NLME approaches for both combination and individual administrations.

Butorphanol Irrelevant Plasma and Urine Concentration (IPC and IUC)

Estimated EPC and IPC values for combination administration (2,692-2,741 pg/ml and 5-6 pg/ml respectively) were approximately half that for individual administration (5,995-6,450 pg/ml and 12-13 pg/ml respectively) see Supplementary Table 7. Plasma DTs were consistent between combination (>47 hours) and individual administration (49 hours) although no definitive value could be given for the combination administration due to the IPC being below the LLOQ. Urine DT for individual administration (57 hours) was similar to combination administration (50 hours).

Intravenous plasma and urine pharmacokinetics for detomidine hydrochloride (10 μg/kg)

The plasma and urine PK profiles for detomidine hydrochloride dosed at 10 μg/kg as a single IV administration are displayed in Figures 3 and 4, respectively, as red symbols and dashed connecting lines. The plasma decay curves appear to follow a single exponential decay. The urine PK curves display only a few time points due to low concentrations of detomidine. The ratio of urine to plasma concentration over the 2 to 6 hour range is approximately 4.

Intravenous plasma and urine pharmacokinetics for detomidine hydrochloride (10 μg/kg) co-administered with 25 μg/kg butorphanol tartrate

The plasma and urine PK profiles for detomidine hydrochloride dosed at 10 μg/kg in combination with 25 μg/kg butorphanol tartrate as an IV administration are displayed in Figures 3 and 4, respectively, as black symbols and solid connecting lines. The plasma decay curves appear to have two phases

of decline, however, the first phase is not explicit. The urine PK curves display limited time points due to low concentrations of detomidine. The ratio of urine to plasma concentration over these time points is approximately 8.

Detomidine Plasma PK Analysis

Table 3 displays the average NCA PK parameters with standard deviations and suggests in this study that detomidine co-administered with butorphanol has a statistically longer half-life ($p<0.05$) but statistically shorter MRT ($p<0.05$) when compared to detomidine dosed on its own. However, there is no significant difference for the parameters clearance and Vss between single and co-administration of detomidine. The most parsimonious NLME model obtained was a simple 1 compartment model, random effects included on clearance only but not volume of distribution and no significant effect of the covariate i.e. individual versus combination dosing. Table 4 shows the outputted PK parameters for the most parsimonious model which were encompassed by the 2.5 and 97.5% confidence intervals of the bootstrap resampling analysis. Clearance values were similar between the NCA and NLME approaches for both combination and individual administrations.

Detomidine Irrelevant Plasma and Urine Concentration (IPC and IUC)

Estimated EPC and IPC values for individual administration (127-132 pg/ml and 0.25-0.26 pg/ml respectively) and combination administration (128-165 pg/ml and 0.27-0.33 pg/ml respectively) were similar (see Supplementary Table 8). The estimated IPC values are sub pg/ml and therefore plasma DT could not be confirmed; > 4 hours for individual administration and > 9 hours for combination administration. Urine DT for individual administration could not be confirmed (> 4 hours), however, a urine DT of 11 hours was confirmed for the combination administration.

Plasma and urine pharmacokinetic profiles for 3-hydroxydetomidine resulting from the metabolism of detomidine hydrochloride

The plasma and urine PK profiles for the metabolite 3-hydroxydetomidine are displayed in Figures 5 and 6. The plasma profiles are characterised by an initial increase followed by a two phase decline and the concentration of the metabolite from the single administration of detomidine appears to be higher than the concentrations resulting from the combination with butorphanol. The urine profiles are characterised by a slow increase in metabolite leading to a flat phase followed by a single phase of decline. The urine concentrations of metabolite from the single administration of detomidine initially appear to be lower than the urine concentrations resulting from the combination with butorphanol, however, after 20 hours post dose the concentrations are similar.

Discussion

The analytical methods applied in the current study are significantly more sensitive than those previously applied for determining the disposition of detomidine and butorphanol in the horse. The LLOQs (supplementary Table 6) for butorphanol, detomidine and 3-hydroxydetomidine in plasma from the current study range between 5 and 10 pg/ml, which are significantly lower than previously reported. The LLOQs (supplementary Table 6) for butorphanol, detomidine and 3-hydroxydetomidine in urine from the current study range between 10 and 100 pg/ml. Compared to plasma, there are fewer existing reports that measure the concentrations of these analytes in urine. However, the urine LLOQs for the detomidine derived analytes in the current study are at least 100-fold lower than reported previously (Machnik et al., 2006). The significantly enhanced analytical sensitivity provided by the methods applied herein permits a lengthened window of detection for all of the analytes. In turn, it is anticipated that this should permit a more accurate assessment of the PK disposition of the drugs at the later time points. Furthermore, the concurrent measurement of plasma and urine concentrations allows for the calculation of plasma to urine drug ratios, which enables urinary drug concentrations to be used as surrogates for plasma concentrations when regulating the use of these medications.

Ideally a three-way cross over study in the same horses should be carried out in order to compare the PK between butorphanol and detomidine single administrations and their co-administration. This would be a very expensive study for any one research centre and there would likely be ethical issues arising from multiple administration of this nature to the same animal in the timescales needed to provide scientifically robust data. A collaboration was therefore put in place via the European Horserace Scientific Liaison Committee (EHSLC) to combine data from three separate studies. Although this is not a cross over study it does allow the finding of any gross changes in the PK between single administration and co-administration.

Both the NCA and NMLE approaches in the herein study suggest that the clearance of butorphanol in combination with detomidine is approximately half that for an individual butorphanol administration. This is further supported by the Knych et al., 2012 study where the clearance for butorphanol dosed individually was estimated to be 690 ±150 ml/hour/kg i.e. approximately twice that of the combination clearance. However, there was no significant difference

in the butorphanol terminal half-lives which were superimposable (Figures 1 and 2) and suggests that the terminal half-life is influenced by a physiological compartment in the horse that can be saturated and represents only a small percentage of the area under the plasma curve (AUC). Despite the butorphanol dose administration being four times greater for a single administration compared to the clinical dose when co-dosed with detomidine the difference in AUC was only 2 fold. This pharmacokinetic advantage probably contributes to the lower dose required for butorphanol to be efficacious in combination with the alpha-2-agonist.

The Toutain and Lassourd (2002) methodology estimates an irrelevant plasma concentration (IPC) for a drug using both the IV therapeutic dose and clearance when dosed individually. The IPC for butorphanol dosed in combination with the alpha-2-agonist is approximately 2 fold lower than that for butorphanol dosed on its own because of differences in both dose and clearance. While the plasma DT can be confirmed for butorphanol dosed individually (51 hours) it cannot be confirmed for combination administration (>47 hours) due to the IPC being below the LLOQ. However, if a plasma screening limit can be set at 10 pg/ml based on risk management then a withdrawal time can be estimated for both butorphanol dosed individually or in combination with detomidine using the NLME model as the model includes BLQ data. A withdrawal time of 72 hours was estimated for a screening limit of 10 pg/ml based upon the predicted tolerance interval representing 95% confidence of the 95^{th} percentile using montecarlo simulation.

For the purposes of detecting butorphanol in horse, analysis in urine gives concentrations that are 730 and 560 times higher than the corresponding concentrations in plasma at pseudo steady-state (Rss) for administration of butorphanol alone and co-administered, respectively. This makes urine a very good matrix for butorphanol screening with similar DT but a 3 fold difference in the IUCs for individual administration and co-administration with detomidine. Pragmatically only one screening limit can be used to control for butorphanol administration, fortunately, the use of 3 ng/ml as a urine screening limit gives the same detection times as determined in Supplementary Table 7. Therefore a detection time of 72 hours will comfortably control for butorphanol administration either individually or in combination with detomidine.

Butorphanol appears to have no significant effect on the pharmacokinetics of detomidine. Whilst there is a statistically shorter plasma half-life for detomidine

dosed on its own at 10 μg/kg compared with detomidine dosed with 25 μg/kg butorphanol there is no statistical difference between the clearance values. Furthermore, the MRT for detomidine single administration is statistically longer compared to the detomidine combination which is inconsistent with an inhibitory drug-drug interaction. This is supported by the NMLE model where the most parsimonious model did not include the covariate for individual versus combination administration.

Based upon the Toutain and Lassourd approach the plasma IPC (and therefore the plasma screening limit) for both detomidine and detomidine dosed in combination with butorphanol will be the same as the clearance values are not statistically different. However, the estimated IPC values are less than 1 pg/ml which is too low for routine screening of a blood sample using current technologies.

Analysis of detomidine in urine gives concentrations that are only 4 (detomidine alone) and 8 (detomidine in combination with butorphanol) times higher than the corresponding concentrations in plasma at pseudo steady-state (Rss). Furthermore, urine data is variable and combined with the relatively low Rss values suggests that urine is also a poor matrix for detomidine detection.

European rules of racing require a minimum of 48 hours between administration and competition and given that detomidine has a very short half-life (< 1 hour), monitoring of parent detomidine is of limited use. However, the 3-hydroxy metabolite of detomidine gives consistent concentrations in plasma and very high concentrations in urine. Therefore, detection of the 3-hydroxy metabolite in urine offers an excellent way forward for the controlling of detomidine administration with a screening limit of 100 pg/ml controlling for at least 35 hours post detomidine administration.

The data herein suggests that either detomidine impacts on the pharmacokinetics of butorphanol or that butorphanol's clearance is dose dependent between 25-100 μg/kg. For the latter scenario, the clearance is smaller at the lower dose which is hard to rationalise, however, a dose response PK study with butorphanol alone would answer this question. For the former scenario, a three-way cross over study in the same horses should be investigated with the butorphanol/detomindine combination although this would be a very expensive study. Alternatively, a relatively cheap in vitro drug-drug interaction study between butorphanol and detomidine using horse microsomes would

confirm or not any pharmacokinetic drug-drug interaction. This research highlights the need to investigate the pharmacokinetics of drugs administered in combination to the horse as potential drug-drug interactions may occur which may have a significant effect on both therapeutic and toxic outcomes as well as leading to false positives in the control of medications within the equine sports industry.

Acknowledgements

The BHA, Irish Horse Racing Board and the FN, German Equestrian Federation are also thanked for funding the project. Staff at the BHA CRS are gratefully acknowledged for their support on administration studies.

Conflict of interest

The authors have no commercial conflict of interests; however, the authors are either employed by, working on behalf of or consulting to a regulatory agency.

Authors' contribution

JB, CH and MM contributed to the analytical development method, validation and sample analysis. LH led the administration study PH and CP performed project management, JS and PH performed manuscript editing. SP co-ordinated the data and performed pharmacokinetic analysis. All authors contributed to the writing of the manuscript, and have read and approved the final manuscript.

ORCID

S.W. Paine http://orcid.org/0000-0001-9443-2311

P.R. Hincks http://orcid.org/0000-0002-0810-9284

References

Arguedas, M. G., Hines, M. T., Papich, M. G., Farnsworth, K. D. and Sellon, D. C. (2008) Pharmacokinetics of butorphanol and evaluation of physiologic and behavioural effects after intravenous and intramuscular administration to neonatal foals. *J Vet Intern Med.* **22,** 1417-1426.

Elfenbein, J. R., Sanchez, L. C., Robertson, S. A., Cole, C. A. and Sams, R. (2009) Effect of detomidine on visceral and somatic nociception and duodenal motility in conscious adult horses. *Veterinary Anaesthesia and Analgesia.* **36,** 162-172.

Grimsrud, K.N., Mama, K.R., Thomasy, S.M. and Stanley, S.D. (2009) Pharmacokinetics of detomidine and its metabolites following intravenous and intramuscular administration in horses. *Equine Vet. J.* **41**, 361–365.

Hannan, C., O'Connor, M. and Barragry, T. (2004) Controlling therapeutic substances-A European harmonised approach: pharmacokinetic studies on eltenac, butorphanol and xylazine in the horse. *Proceedings of the 15th International Conference of Racing Analysts and Veterinarians*, Dubai. pp 473–480.

Hubbell, J. A. E., Sams, R. A., Schmall, L. M., Robertson, J. T., Hinchcliff, K. W. and Muir, W. W. (2009) Pharmaciokinetics of detomidine administered to horses at rest and after maximal exercise. *Equie Vet J.* **41** (5), 419-422.

Knych, H. K., Casbeer, H. C., McKemie, D. S. and Arthur R. A. (2012) Pharmacokinetics and pharmacodynamics of butorphanol following intravenous administration to the horse. *J. vet. Pharmacol. Therap*. 36, 21–30.

Machnik, M., Tobias, S., Hammer, I., Kietzmann, M., Due, M., Weinberger, T., Schenk, I. and Schanzer, W. (2006) Controlling theraputic substances-A European harmonised approach: pharmacokinetic study on the α_2-agonists romifidine and detomidine in horses. *Proceedings of the 16th International Conference of Racing Analysts and Veterinarians*, Tokyo. pp 421–429.

Sellon, D.C., Papich, M.G., Remund, B. and Palmer, L. (2008) Pharmacokinetics of butorphanol in horses after intramuscular injection. *J. Vet. Pharmacol. Therap*. **32**, 62-65.

Toutain, P.L. and Lassourd, V. (2002) Pharmacokinetic/pharmacodynamic approach to assess irrelevant plasma or urine drug concentrations in postcompetition samples for drug control in the horse. *Equine Vet. J.* **34**, 242–9.

Table 1: Pharmacokinetic parameters for butorphanol tartrate dosed at 100 μg/kg as a single administration and at 25 μg/kg in combination with 10 μg/kg detomidine (D) hydrochloride

Regimen	CL (mL/hr/kg)	Vd_Z (L/kg)	$T_{1/2}$ (hr)	MRT (hr)	V_{ss} (L/kg)
100 μg/kg	646 ± 137	5.07 ± 2.48	5.21 ± 1.56	1.45 ± 0.48	0.97 ± 0.45
25 μg/kg (D)	380 ± 86*	2.95 ± 0.38	5.43 ± 0.44	2.63 ± 0.56*	1.01 0.36

*$p<0.05$

Table 2: NMLE three compartment pharmacokinetic parameters for butorphanol tartrate dosed at 100 μg/kg as an individual administration and at 25 μg/kg in combination with 10 μg/kg detomidine hydrochloride. V1, V2, V3 represent the volumes of the central, second and third compartments, respectively. CL1 represents the clearance from the central compartment and CL2 and CL3 represent the distribution clearance between the central compartment and second and third compartments, respectively.

Parameter	Typical Value (TV)	Post hoc mean ± sd	
		Individual Administration	Combination Administration
V1 (mL/kg)	279	276±90.3	322±101
V2 (mL/kg)	471	559±339	479±137
V3 (mL/kg)	221	219±13.4	225±15.8
CL1 (mL/hr/kg)	707	695±118	387±69.7
CL2 (mL/hr/kg)	75	87.2±53.2	77.1±25.8
CL3 (mL/hr/kg)	908	909±36.4	105±5.3

Table 3: NCA pharmacokinetic parameters for detomidine hydrochloride dosed at 10 μg/kg as a single administration and in combination with 25 μg/kg butorphanol tartrate (B).

Regimen	CL (mL/hr/kg)	Vd_Z (L/kg)	$T_{1/2}$ (hr)	MRT (hr)	V_{ss} (L/kg)
10 μg/kg	3278 ± 1412	2.76 ± 1.36	0.57 ± 0.06	0.67 ± 0.11	2.32 ± 1.30
10 μg/kg (B)	2519 ± 630	2.52 ± 0.53	0.70 ± 0.11*	0.56 ± 0.07*	1.39 ± 0.26

*$p<0.05$

Table 4: NMLE one compartment pharmacokinetic parameters for detomidine hydrochloride dosed at 10 μg/kg as an individual administration and in combination with 25 μg/kg butorphanol tartrate. V and CL represent the volume of distribution and clearance, respectively.

Parameter	Typical Value (TV)	Post hoc mean ± sd	
		Individual Administration	Combination Administration
V (L/kg)	2.55	2.55	2.55
CL (mL/hr/kg)	3160	3159±335	3252±147

Figure Legends

Figure 1: Plasma pharmacokinetic profiles for butorphanol dosed at 100 μg/kg as a single administration (red dashed line) and at 25 μg/kg in combination with 10 μg/kg detomidine hydrochloride (black solid line)

Figure 2: Urine pharmacokinetic profiles for butorphanol dosed at 100 μg/kg as a single administration (red dashed line) and at 25 μg/Kg in combination with 10 μg/Kg detomidine hydrochloride (black solid line)

Figure 3: Plasma pharmacokinetic profiles for detomidine dosed at 10 μg/kg as a single administration (red dashed line) and in combination with 25 μg/Kg butorphanol tartrate (black solid line)

Figure 4: Urine pharmacokinetic profiles for detomidine dosed at 10 μg/kg as a single administration (red dashed line) and in combination with 25 μg/kg butorphanol tartrate (black solid line)

Figure 5: Plasma pharmacokinetic profiles for 3-hydroxy detomidine resulting from the metabolism of detomidine hydrochloride dosed at 10 μg/kg as a single administration (red dashed line) and in combination with 25 μg/kg butorphanol tartrate (black solid line)

Figure 6: Urine pharmacokinetic profiles for 3-hydroxy detomidine resulting from the metabolism of detomidine hydrochloride dosed at 10 μg/kg as a single administration (red dashed line) and in combination with 25 μg/kg butorphanol tartrate (black solid line).

Supplementary Information

Title: The intravenous pharmacokinetics of butorphanol and detomidine dosed in combination compared with individual dose administrations to exercised horses

Short Running Title: Intravenous horse pharmacokinetics butorphanol/ detomidine combination

Authors: Stuart W. Paine[1], Jane Bright[2], James P. Scarth[2], Pamela R. Hincks[2], Clive M. Pearce[2], Colette Hannan[3], Marc Machnik[4], Lynn Hillyer[5,6]

[1]School of Veterinary Medicine and Science, University of Nottingham, College Road,
Sutton Bonington, Leicestershire, LE12 5RD, UK
[2] LGC Ltd, Newmarket Road, Fordham, Cambridgeshire, CB7 5WW, UK
[3] BHP Racing Laboratory, New Road, Thomondgate, Limerick, Ireland
[4] Institute of Biochemistry, German Sport University, D50933 Cologne, Germany
[5] British Horse Racing Authority, 75 High Hoborn, London, WC1V 6LS, UK
[6]Current address: Irish Horseracing Regulatory Board, The Curragh, Kildare, Co Kildare, Irela

Supplementary table 1 – Summary of the plasma and urine sample times post administration for butorphanol dosed individually in each horse (Irish Study)

Horse 1	Horse 2	Horse 3	Horse 4	Horse 5	Horse 6	Horse 7	Horse 1	Horse 2	Horse 3	Horse 4	Horse 5	Horse 6	Horse 7
Plasma	**Plasma**	**Plasma**	**Plasma**	**Plasma**	**Plasma**	**Plasma**	**Urine**	**Urine**	**Urine**	**Urine**	**Urine**	**Urine**	**Urine**
Time (hr)	Time (hr)	Time (hr)	Time (hr)	Time (hr)	Time (hr)	Time (hr)	Time (hr)	Time (hr)	Time (hr)	Time (hr)	Time (hr)	Time (hr)	Time (hr)
0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.83	6.5	1.47	0.05	2	1	0.65
0.08	0.08	0.08	0.08	0.08	0.08	0.08	2.83	14.17	8	0.5	5	1.58	1.25
0.17	0.17	0.17	0.17	0.17	0.17	0.17	6.67	21.25	19.17	1.22	25.83	3.25	3.17
0.25	0.25	0.25	0.25	0.25	0.25	0.25	11.5	25.75	24.08	3	36.75	4.42	4.25
0.3	0.3	0.3	0.3	0.3	0.3	0.3	18	28.5	27.5	6.92	49.75	9.3	7.5
1	1	1	1	1	1	1	27.5	31	30.58	10.08	54	11.67	9.25
2	2	2	2	2	2	2	31.75	54.5	48	13.42	71.75	18.17	12.3
4	4	4	4	4	4	4	56.5	56.25	53.5	20	83.25	22.58	22.47
8	8	8	8	8	8	8	76	73.5	55.75	23.92	97.75	25.08	24
12	12	12	12	12	12	12	83.5	77.75	78.5	25.92	108.25	27.67	24.83
24	24	24	24	24	24	24	99	98	85.25	28.5	120.25	47	26.25
30	30	29	29	28	28	28	110.5	101	96.75	47.75	122.5	51.5	27.75
32	32	31	31	30	30	30	123	104.25	106.75	52.75	124.75	71.25	48.5
50.5	48.75	48.75	48.75	48.75	48.75	48.75	128.5	125.75	123.25	72	143.25	75.5	51.75
74	72	72	72	72	72	72	146	146	125.5	77	146.75	95.25	71.25
98	96	96	96	96	96	96	153.75	148	147	97.25		98	75.25
122	120	120	120	120	120	120		149	153	98.5		100.25	96.25
145	144	144	144	144	144	144				99.5		119.5	97.75
										101		121	100
										120.75		122.5	119.75
										123.5		124.25	124
										124.75		143.25	144.5
										144.75		145.25	147.5
										147.25		146.5	
										148.75		147.75	

Supplementary table 2 – Summary of the plasma and urine sample times post administration for detomidine dosed individually in each horse (German Study)

All horses	Horse 207	Horse 208	Horse 215	Horse 217	Horse 229	Horse 257	Horse 281	Horse 282
Plasma	**Urine**	**Urine**	**Urine**	**Urine**	**Urine**	**Urine**	**Urine**	**Urine**
Time (hr)	Time (hr)	Time (hr)	Time (hr)	Time (hr)	Time (hr)	Time (hr)	Time (hr)	Time (hr)
0	0	0	0	0	0	0	0	0
1	2.62	2.63	2.6	2.58	2.57	2.53	2.57	2.47
2	4.98	5.03	5.45	5.13	5.05	5.25	4.2	4.28
4	7.82	7.83	7.68	7.75	8.07	8.25	5.25	7.87
8	11.37	11.58	11.27	11.58	1.33	11.42	11.05	11
12	24.48	24.87	24.52	25	25.2	25.42	25.75	25.5
24	35.48	35.83	35.35	35.55	35.47	35.52	35.18	35.1
36	48.4	48.65	48.38	48.72	49	49.13	49.42	49.18
48	59.57	59.98	59.43	59.65	59.67	59.67	59.28	59.2
	71.87	72.83	73.12	72.53	74.5	72.08	71.65	71.78
	82.98	83.42	83.18	83.12	83	83.17	82.7	82.92
	95.88	96.2	95.88	96.2	96.95	96.25	96.35	96.42

Supplementary table 3 – Summary of the plasma and urine sample times post administration for combination of butorphanol and detomidine dosed in each horse (UK Study)

Horse 13/11	Horse 14/11	Horse 15/11	Horse 16/11	Horse 17/11	Horse 18/11	Horse 13/11	Horse 14/11	Horse 15/11	Horse 16/11	Horse 17/11	Horse 18/11
Plasma	**Plasma**	**Plasma**	**Plasma**	**Plasma**	**Plasma**	**Urine**	**Urine**	**Urine**	**Urine**	**Urine**	**Urine**
Time (hr)	Time (hr)	Time (hr)	Time (hr)	Time (hr)	Time (hr)	Time (hr)	Time (hr)	Time (hr)	Time (hr)	Time (hr)	Time (hr)
-144.08	-142.08	-96.33	-96.32	-89.18	-89.35	-142.17	-138.25	-112.92	-95.47	-22.08	-94
-113.17	-113.33	-72.7	-72.92	-17.33	-17.58	-22.82	-24.08	-90.08	-63.92	-18.17	-21.83
-0.3	-0.22	-0.33	-0.22	-0.33	-0.12	2.48	1.42	1.75	1	1.05	1.78
0.25	0.25	0.25	0.28	0.25	0.25	3.33	3.25	2.5	2.12	1.42	5.08
1	1	1	1.03	1	1	4.75	5.5	3.67	4.28	1.92	11.33
2	2	2	2.07	2.03	1.97	5.8	6.62	6.92	5.87	3	18.83
3	3	3	3.03	3	3	6.77	9.08	9.67	7.12	5.55	23.13
4.08	4.07	4	4.03	4.08	4.08	9.25	11.08	13.17	9.45	6.25	29
5.08	5.08	5	5.03	5.08	5	11.08	14.75	14.58	10.45	8.08	38.83
6	6	6	6.03	6	6	14.75	18.75	18.83	12.45	9.5	50.17
7	7	7	7.03	7.03	7	19.17	23.17	22.83	15.45	10.92	55.17
8	8	8	8.08	8	8	22.67	25.67	26.33	19.62	12.83	74.15
9	9	9	9.08	9	9	24.17	27.25	30.33	22.87	20.17	78.58
11	11	11	11.08	11	11	25.55	30.67	36.58	23.7	25.8	96.7
13	13	13	13.03	13	13	28	33.08	39.75	24.45	28.08	103.17
15	15	15	15.03	15	15	29.45	37.08	46.67	25.12	31.25	120.75
19	19	19	19.03	19	19	30.83	38.92	48.17	26.37	38.5	126.83
23	23	23	23.03	23.03	23	32.83	48.08	54.33	29.37	48.33	143.83
25	25.08	25	25.03	25	25	35.75	54.08	72.67	30.28	54.42	150.72
27	27	27	27.03	27	27	37.08	71.42	79.17	32.53	72.5	169.33
29	29	29	29.03	29.08	29.08	38.5	77	96.83	36.68	77.42	193.75
31	31	31	31.03	31	31	48.25	96.67	105.5	39.12	97.25	222.65
33	33	33	33.03	33	33	53.83	103.92	118.42	39.62	103.42	242.25
35	35	35	35.03	35	35	71.5	120.08	126.67	46.62	119.42	
37	37	37	37.12	37	37	77	127.42	147	48.4	125.75	
39	39	39	39.08	39	39	97.25	145.22	151.25	54.53	144.42	
46.83	46.92	46.67	46.68	46.67	46.65	102.42	150.17	170.8	72.95	149.67	
54.42	54.5	54.72	54.77	54.67	54.7	120	174.08	194.83	78.2	169.83	
70.5	70.67	70.67	70.7	70.67	70.7	126.33	193.58	222.92	95.12	194.08	
78.5	78.5	78.67	78.7	78.5	78.5	145.42	217.75	242.25	102.95	225.25	
94.7	94.68	94.67	94.7	94.67	94.83	150.33	241.17		104.87	242.83	
102.5	102.48	102.67	102.7	102.83	102.92	174.25			118.87		
118.5	118.48	118.67	118.68	118.97	118.75	191.67			126.87		
126.5	126.48	126.67	126.68	126.67	126.68	217.65			144.53		
143.08	143.12	142.83	142.72	143.08	143.08	241.08			149.87		
150.47	150.45	150.92	150.95	150.75	150.58				167.12		
									192.78		
									214.28		
									238.45		

Supplementary table 4 – Summary of the sample preparation/extraction methods used by each laboratory

Germany	Ireland		United Kingdom	
Plasma and urine (detomidine and 3-hydroxydetomidine)	**Plasma** (butorphanol)	**Urine** (butorphanol)	**Plasma** (butorphanol, detomidine, 3-hydroxydetomidine)	**Urine** (butorphanol, detomidine, 3-hydroxydetomidine)
To 5 ml of plasma/urine: - Add medetomidine & D_4-3-hydroxydetomidine internal standards. - Add 0.4 mL 4M sodium acetate buffer + adjust pH to 5.2. - Add 50 µl Helix pomatia + heat for 1 hour at 50ºC. - Add 0.5 ml 0.8M sodium phosphate (pH7), shake and then centrifuge for 5 mins. - SPE using Oasis HLB (6 ml, 200 mg). - Condition with methanol - Condition with water. - Apply sample. - Wash with water. - Wash with 10% methanol in water. - Elute with methanol. - Evaporate, reconstitute in 1 ml TBME + 0.4 ml 0.06M HC, shake and then centrifuge for 5 mins. - Remove the aqueous layer to LCMS vials.	*To 4 ml of plasma:* - Add meperidine internal standard. -Add phosphate buffer 0.1 M pH 5.5 and centrifuge for 10 mins. - SPE using Bond Elut Certify (6 ml, 300mg). - Condition with methanol - Condition with phosphate buffer. - Apply sample. - Wash with water. - Wash with acetate buffer. - Wash with methanol. - Elute with ethyl acetate:triethylamine. - Evaporate, reconstitute in 50µl methanol + transfer to an LCMS vial.	*To 3 ml of urine:* - Add meperidine internal standard. -Add phosphate buffer 0.1 M pH 5.5, β-glucuronidase from helix pomatia & protease+ adjust pH to 6.5. - Heat at 55 ºC for 1 hour then centrifuge for 10 mins. - SPE using Bond Elut Certify (6 ml, 300mg). - Condition with methanol - Condition with phosphate buffer. - Apply sample. - Wash with water. - Wash with acetate buffer. - Wash with methanol. - Elute with ethyl acetate:triethylamine. - Evaporate, reconstitute in 100µl methanol + transfer to an LCMS vial.	*To 1 ml of plasma:* - Add medetomidine & D_4-3-hydroxydetomidine internal standards. - Add helix pomatia. - Heat overnight at 45 ºC. - Add 600 µl 0.1M NaOH in water. - Add 3 ml 15% chloroform in TBME. - Mix on a rotary mixer for 30 mins then centrifuge for 10 mins. - Transfer the organic layer to clean tubes, evaporate + reconstitute in 5 µl IPA + 100 µl 10 mM ammonium formate, transfer to LCMS vials + centrifuge for 10 mins.	*To 2 ml urine:* - Add medetomidine & D_4-3-hydroxydetomidine internal standards. - Add acetate buffer 1 M pH 4.7, β-glucuronidase from helix pomatia & pancreatin. - Heat overnight at 45 ºC then centrifuge for 10 mins. - SPE using Phenomenex Strata XC (3 ml, 60 mg). - Condition with methanol - Condition with water. - Apply sample. - Wash with acetate buffer 0.1 M pH 9.0. - Wash with water. - Wash with.1 M HCl. - Wash with methanol. - Elute with 80 % ethyl acetate: 17 % isopropanol and 3 % ammonia (v/v). - Evaporate + reconstitute in 5 µl acetonitrile and 100 µl 10 mM ammonium formate, transfer to LCMS vials + centrifuge for 10 mins.

Supplementary Table 5 – Summary of the instrumental conditions used by each laboratory

	Germany	Ireland	United Kingdom
Instrumental platform	HPLC = Agilent 1200 MS = AB Sciex 4000 Q-Trap	HPLC = Agilent 1100 MS = Agilent MSD XCT Ion Trap	HPLC = Waters Acquity MS = AB Sciex 5500 Q-Trap
HPLC conditions	Column = Phenomenex Gemini 3µ C6-Phenyl 150 x 4.6 mm. Mobile phase A = MeCN and B = 5mM ammonium acetate (pH 3.5). Flow = 0.5 ml/min. Gradient = 0% A at 0 mins, held at 0% until 1 mins, to 100% at 10 mins, held at 100% until 13.5 mins.	Column = Phenomenex Kinetex PFP 100 × 2.1mm, 2.6µm Mobile phase A = 10mM ammonium acetate and B = MeCN Flow = 0.3 ml/min Gradient = 27% B at 0 mins, rising to 51% at 6 mins, to 95% at 7 mins, to 27% at 11.1 mins, held at 27% until 13 mins.	Column = Waters HSS T3 100 x 2.1 mm, 1.8 µm. Mobile phase A = MeCN & B = 10mM ammonium formate in water (pH 7.0). Flow = 0.85 ml/min. Gradient = 0% A at 0 mins, rising to 3.8% at 1.5 mins, to 6.0% at 1.51 mins, to 20.0% at 2.8 mins, to 24.0% at 2.81 mins, to 30.0% at 4.2 mins, to 90.0% at 4.21 mins, to 99.9% at 6.20 mins, held at 99.9% until 6.70 mins, to 0% at 6.71 mins, held at 0% until 7.0 mins.
MS/MS conditions	Turboionspray ionisation in positive mode. Temperature = 450°C. Ionspray voltage = 5500. Detomidine = *m/z* 187 to 81, CE = 29. 3-OH-detomidine = *m/z* 203 to 185, CE = 21. Medetomidine (internal standard for detomidine) = *m/z* 201 to 68, CE = 49. D_4-3-OH-detomidine (internal standard for 3-OH-detomidine) = *m/z* 207 to 81, CE = 35.	Electrospray ionisation in positive mode. Temperature = 350°C Capillary Voltage = 4000 Butorphanol = 328 to 310 (amplitude = 1.00) to 310 , 282, 242 (amplitude = 0.47) (combined EIC of all 3 used for quantitation) Meperidine (internal standard) = 248 to 220, 174(amplitude = 0.94) (combined EIC of 2 ions used for quantitation)	Turboionspray ionisation in positive mode. Temperature = 650°C. Ionspray voltage = 5500. Butorphanol = *m/z* 328 to 185, CE = 47. Detomidine = *m/z* 187 to 81, CE = 13. 3-OH-detomidine = *m/z* 203 to 81, CE = 70 (urine) & 17 (plasma). Medetomidine (internal standard for butorphanol & detomidine) = *m/z* 201 to 95, CE = 25. D_4-3-OH-detomidine (internal standard for 3-OH-detomidine) = *m/z* 207 to 81, CE = 31.

Supplementary Table 6 – Validated calibration ranges from each laboratory

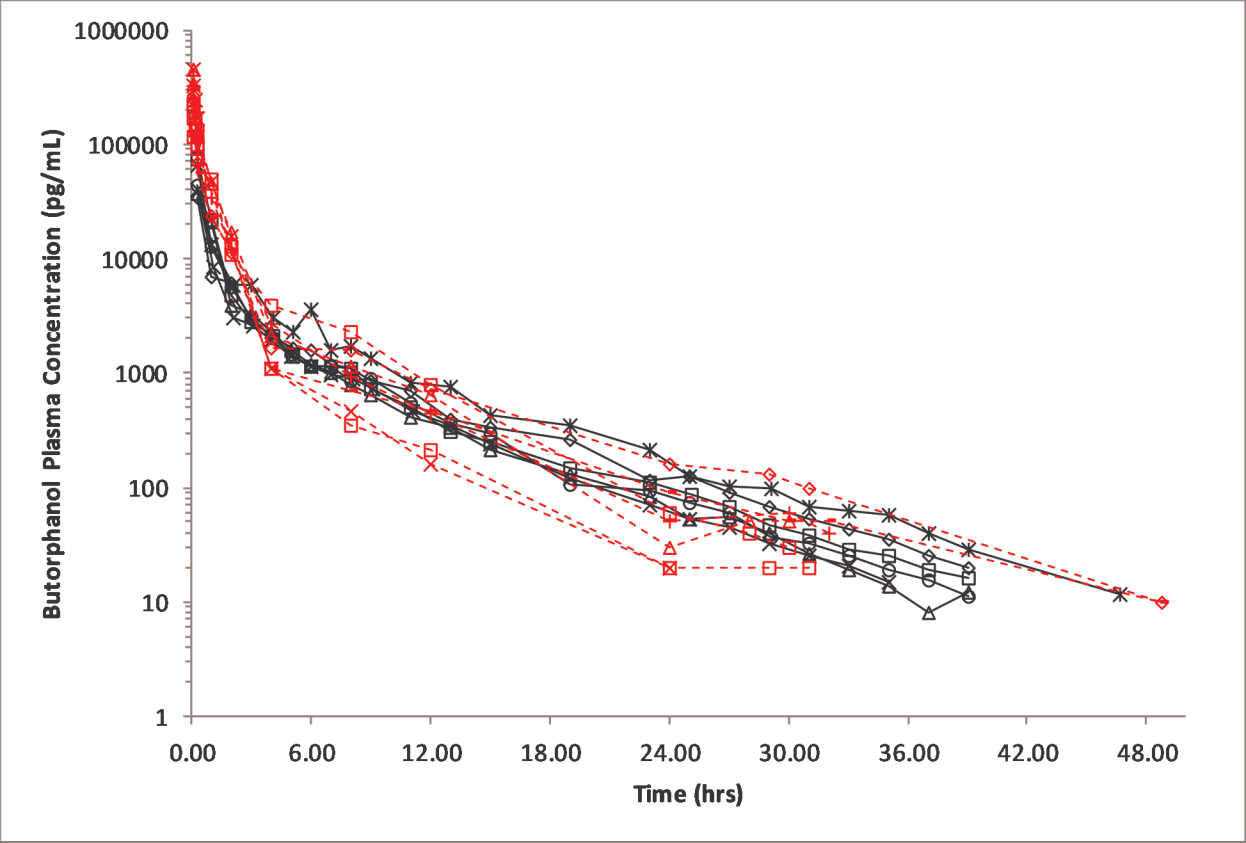
Validated calibration ranges and highest validated dilution factors where relevant (calibration line weighting in brackets)						
Analyte	**Germany (pg/ml)**		**Ireland (pg/ml)**		**United Kingdom (pg/ml)**	
	Plasma	**Urine**	**Plasma**	**Urine**	**Plasma**	**Urine**
Butorphanol	N/A	N/A	10 – 1,500 (no weighting) + 10-fold dilution	100 – 5,000 (no weighting) + 10-fold dilution	10 - 10,000 ($1/x^2$) + 1000-fold dilution	100 - 10,000 ($1/x^2$) + 1000-fold dilution
Detomidine	10 - 2,500 (1/x)	100 - 25,000 (1/x)	N/A	N/A	5 - 10,000 ($1/x^2$) + 1000-fold dilution	10 - 10,000 ($1/x^2$) + 1000-fold dilution
3-Hydroxydetomidine	10 - 2,500 ($1/x^2$)	100 - 25,000 and 25,000 – 200,000 (both lines $1/x^2$)[a]	N/A	N/A	5 - 10,000 ($1/x^2$) + 100-fold dilution	100 - 10,000 ($1/x^2$) + 1000-fold dilution

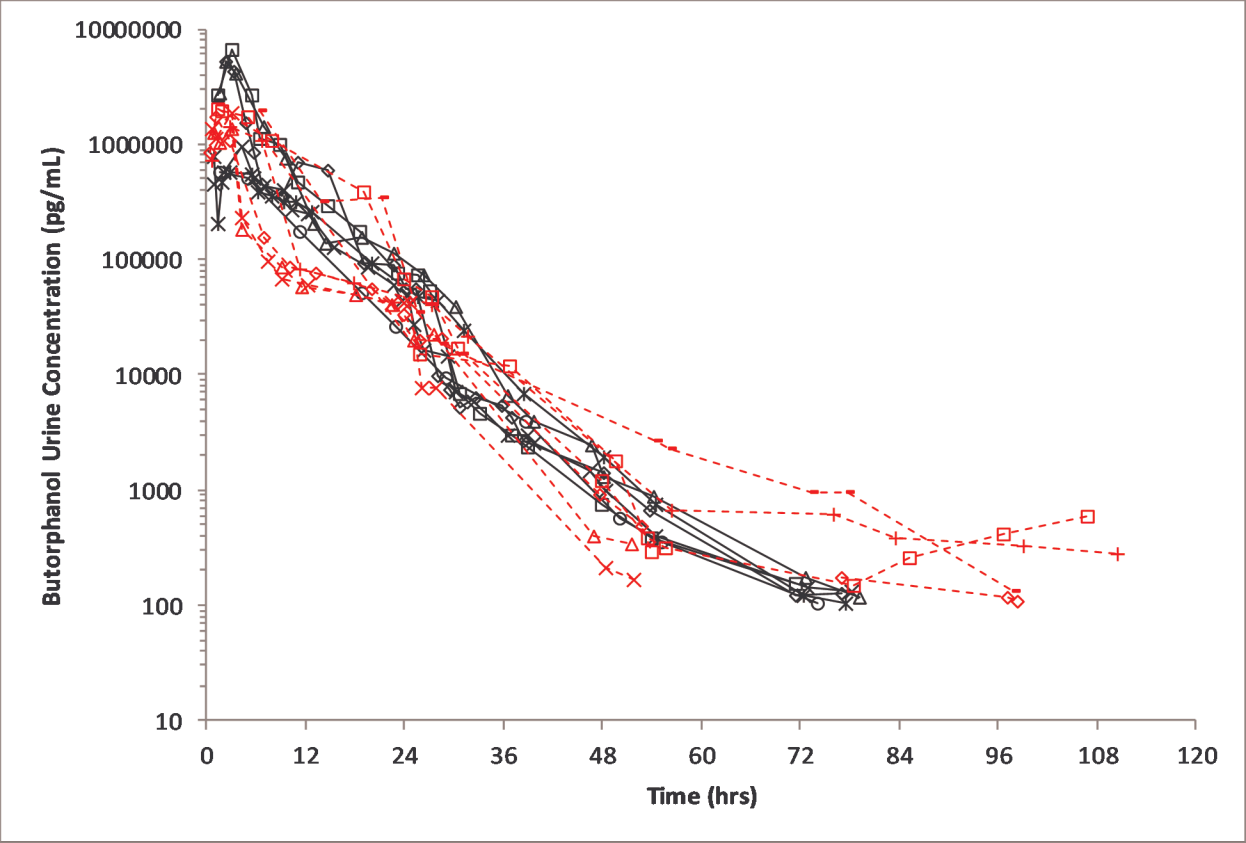
Supplementary Table 7: Estimated EPCs for individual versus combination administration by dividing the dose of butorphanol by the mean plasma clearance over a 24 hour period determined by either an NCA or NMLE (posthoc) approach. The IPCs were determined by dividing the EPC by a factor of 500 and the IUC determined by multiplying the IPC by the pseudo steady-state ratio of urine to plasma concentration (Rss). The detection times (DT) were determined by the time post dose where all horses had concentrations below the IPC/IUC.

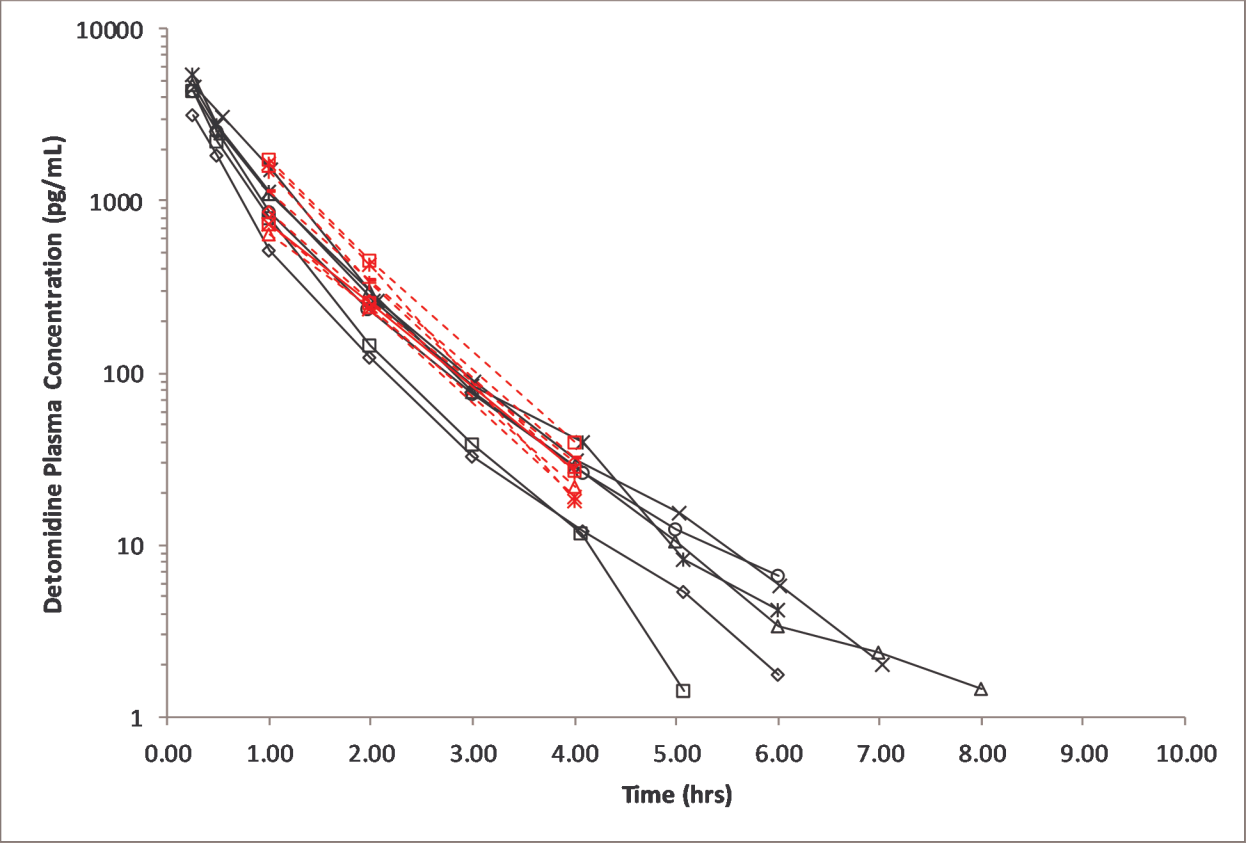
Parameter	Individual Administration		Combination Administration	
	NCA	NLME	NCA	NLME
EPC (pg/mL)	6450	5995	2741	2692
IPC (pg/mL)	13	12	6	5
Rss	730	730	560	560
IUC (pg/mL)	9490	8760	3080	3015
Plasma DT (hrs)	49	49	>47	>47
Urine DT (hrs)	57	57	50	50

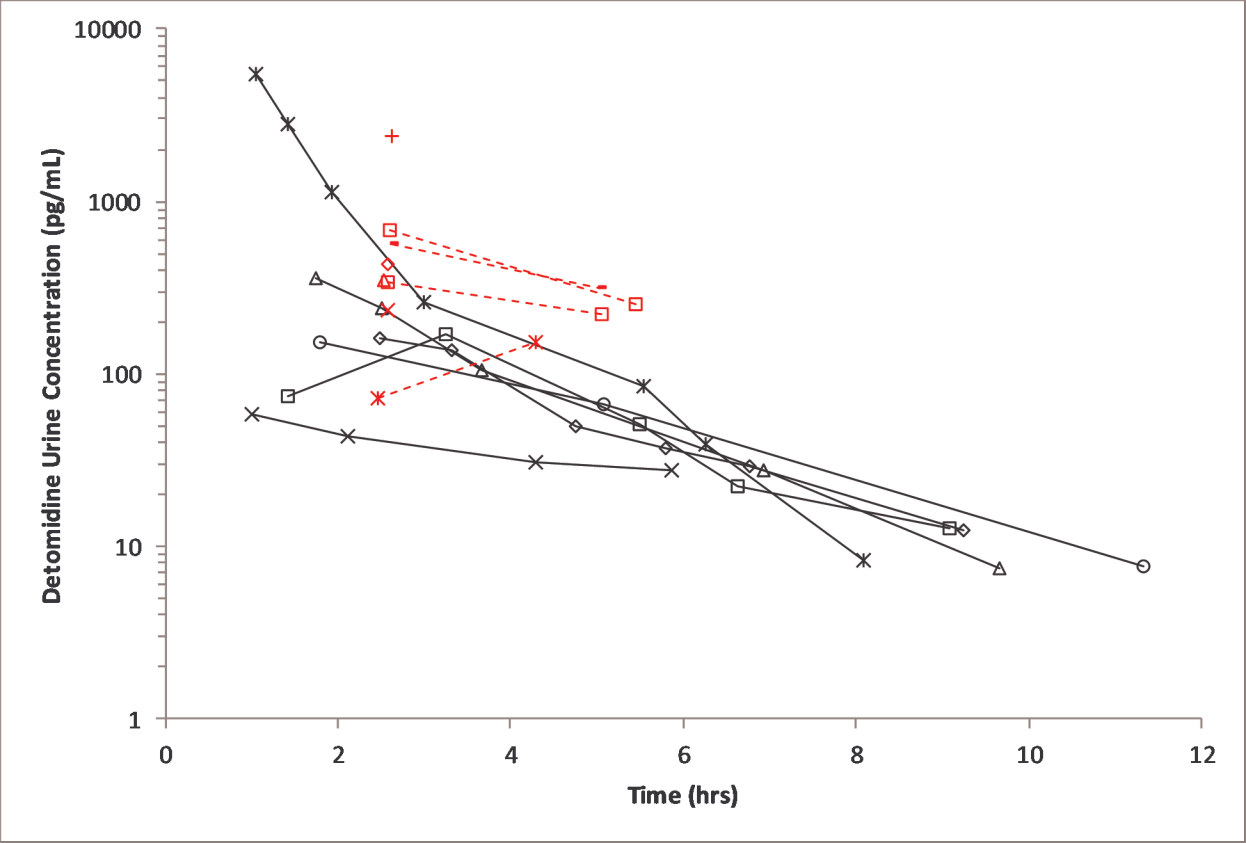
Supplementary Table 8: Estimated EPCs for individual versus combination administration by dividing the dose of detomidine by the mean plasma clearance over a 24 hour period determined by either an NCA or NMLE (posthoc) approach. The IPCs were determined by dividing the EPC by a factor of 500 and the IUC determined by multiplying the IPC by the pseudo steady-state ratio of urine to plasma concentration (Rss). The detection times (DT) were determined by the time post dose where all horses had concentrations below the IPC/IUC.

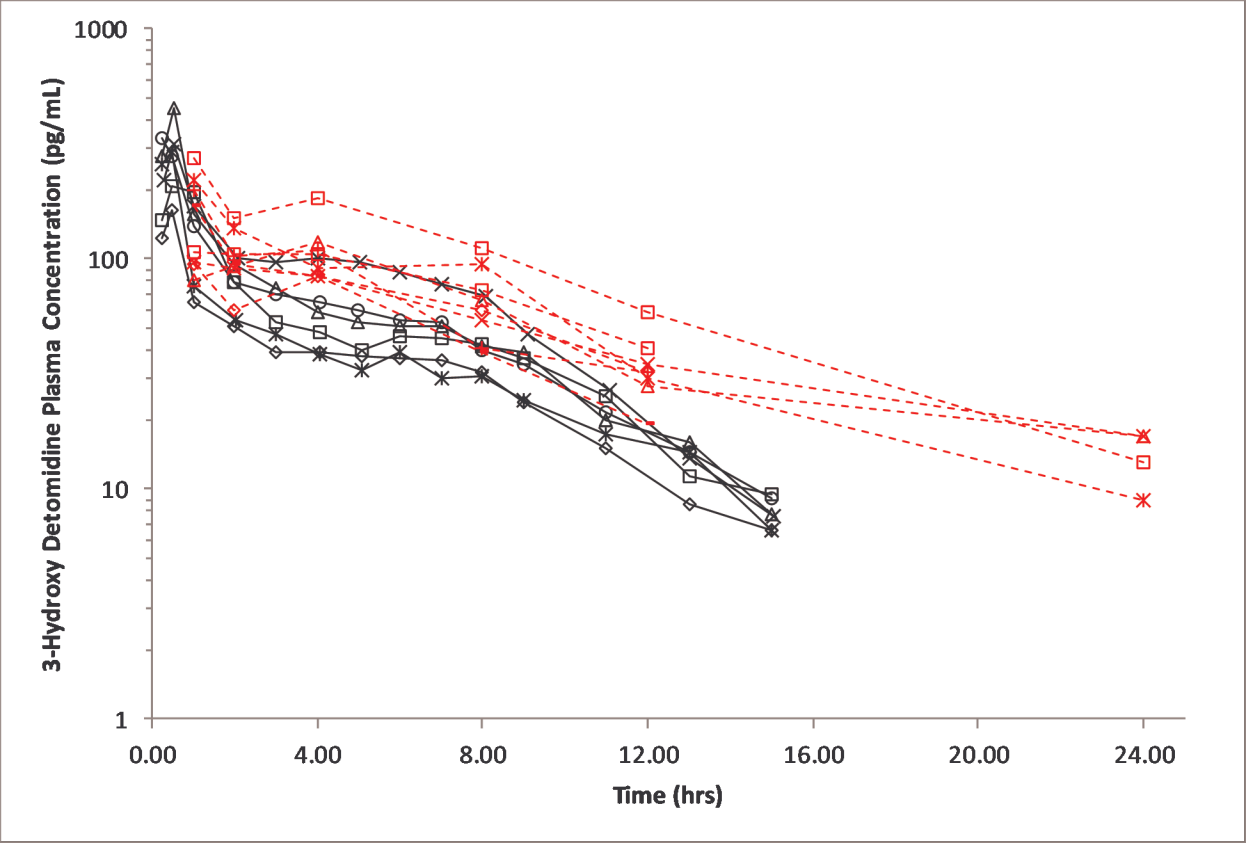
Parameter	Individual Administration		Combination Administration	
	NCA	NLME	NCA	NLME
EPC (pg/mL)	127	132	165	128
IPC (pg/mL)	0.25	0.26	0.33	0.27
Rss	4	4	8	8
IUC (pg/mL)	1.02	1.04	2.64	2.05
Plasma DT (hrs)	>4	>4	>9	>9
Urine DT (hrs)	>4	>4	11	11

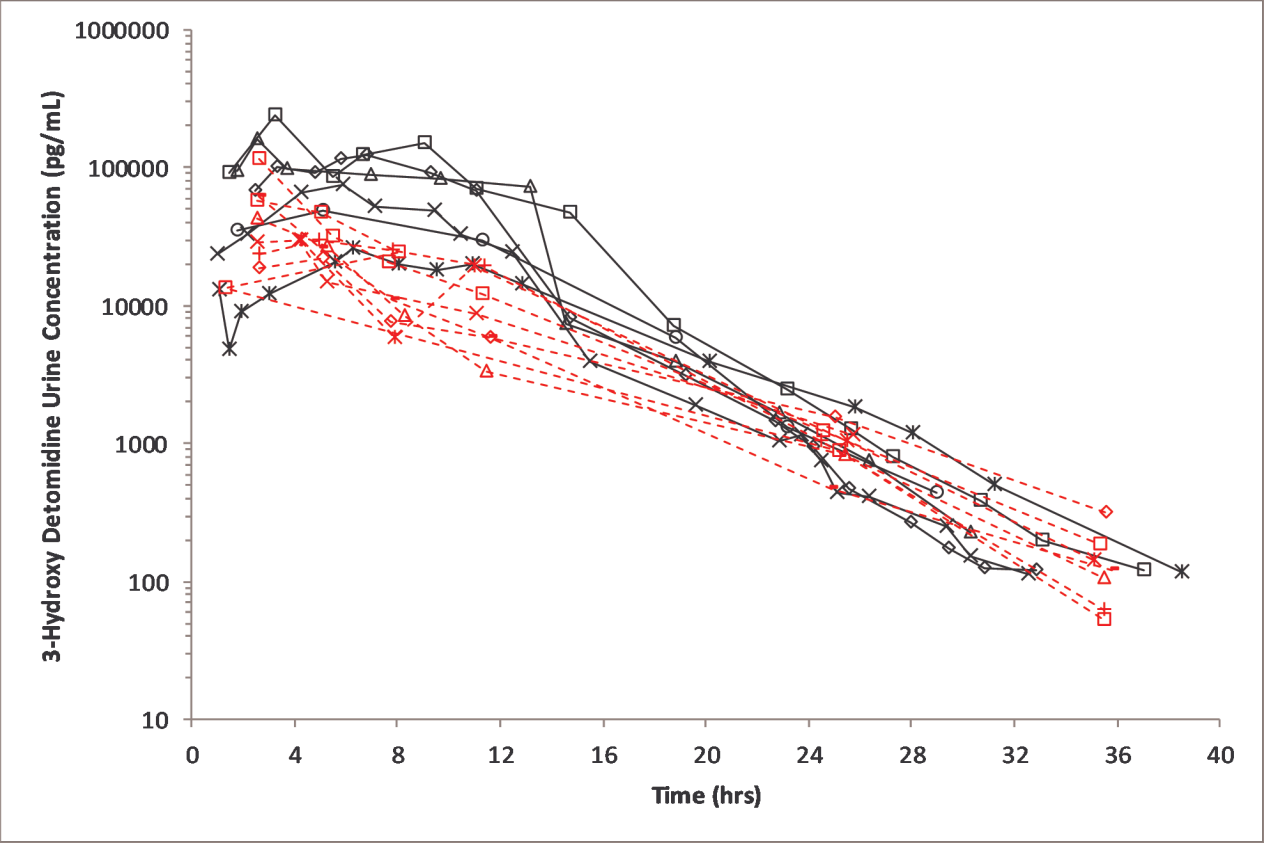

Butorphanol Plasma Concentration (pg/mL)
1000000
100000
10000
1000
100
10
1
0.00
6.00
12.00
18.00
24.00
30.00
36.00
42.00
48.00
Time (hrs)


Butorphanol Urine Concentration (pg/mL)
10000000
1000000
100000
10000
1000
100
10
0
12
24
36
48
60
72
84
96
108
120
Time (hrs)


Detomidine Plasma Concentration (pg/mL)
10000
1000
100
10
1
0.00
1.00
2.00
3.00
4.00
5.00
6.00
7.00
8.00
9.00
10.00
Time (hrs)


10000
1000
100
10
1
Detomidine Urine Concentration (pg/mL)
0
2
4
6
8
10
12
Time (hrs)


1000
100
10
1
3-Hydroxy Detomidine Plasma Concentration (pg/mL)
0.00
4.00
8.00
12.00
16.00
20.00
24.00
Time (hrs)


1000000
100000
10000
1000
100
10
3-Hydroxy Detomidine Urine Concentration (pg/mL)
0
4
8
12
16
20
24
28
32
36
40
Time (hrs)