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**Title:** Effect of feeding on the pharmacokinetics of vilazodone in dogs

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**Running head:** Pharmacokinetics of vilazodone in dogs

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**ABSTRACT**

Vilazodone (VLZ) is a drug approved for the treatment of major depressive disorder in humans but no data are available for dogs. The present study aimed to evaluate the pharmacokinetics of a single oral 40 mg dose of VLZ in healthy Labrador dogs (n=6) in fasted and fed conditions. Dogs were randomly divided in two (n=3) groups in a cross-over study design (2x2). Group I was administered with VLZ at 40 mg/dog after fasting over-night. Group II was fed prior to and after administration of the same dose. A two-week wash-out period was observed. Plasma samples collected underwent LC-MS/MS analysis.

VLZ concentrations were quantified in dogs' plasma in two different windows of time: 30 min to 10 h for the fasted group and 4 h to 35 h for the fed group. The values for  $t_{1/2\lambda z}$  were statistically different between the groups (fed,  $4.6 \pm 1.1$  h vs fasted,  $1.7 \pm 0.2$  h).  $T_{max}$  drastically changed between the groups (fed, 10 h vs fasted, 1.5 h), while  $C_{max}$  did not significantly vary (fed,  $39.4 \pm 5.6$  ng/mL vs fasted,  $38.7 \pm 4.8$  ng/mL). The AUC value was always statistically higher in the fed group. As a result, the average relative oral fasted bioavailability of VLZ was low,  $28.8 \pm 6.1\%$ . In conclusion, feeding can affect the pharmacokinetics of VLZ in the dog.

*Key words:* Vilazodone; Dogs; unfed-fed; LC-MS/MS

## INTRODUCTION

In the last two decades psychiatric disorders such as depression, anxiety, mania, obsession, sleep deprivation and hyperactivity have been recognized to affect not only human beings but also pets (Overall, 2013). Consequently, drugs labelled for humans have started to be clinically used off-label in pets. Their administrations were often based on the dose regimen for men without any specific pharmacokinetic or pharmacodynamic rationale (Crowell-Davis and Mattos de Souza Dantas, 2019). The use of an active ingredient in a new animal species needs a step wise approach with pharmacokinetic, pharmacodynamic and safety tests, in order to avoid likely complications for the patient or a therapy failure (Lavy et al., 2011; Giorgi et al., 2012a, 2012b; Giorgi and Yun, 2012).

Vilazodone (VLZ) is a phenylpiperazine chemical derivative approved for the treatment of major depressive disorder in humans (Boinpally et al., 2014). VLZ garnered FDA approval for treating major depressive disorders in 2011 on the basis of its antidepressant efficacy and general tolerability profile (<http://www.drugs.com/history/vilazodone.html>). This active ingredient combines two mechanisms in a single drug, namely the selective serotonin reuptake inhibitors (SSRI) with 5HT<sub>1A</sub> receptor partial agonist actions, and a serotonin partial agonist reuptake inhibitor (SPARI) (Fig. 1). Specifically, this agent increases the availability and activity of the neurotransmitter serotonin and its neuro pathways. VLZ blocks the serotonin reuptake pump (serotonin transporter or SERT), desensitizes serotonin receptors (especially 5HT<sub>1A</sub> autoreceptors), and therefore presumably increases serotonergic neurotransmission. Its partial agonist actions at presynaptic somatodendritic 5HT<sub>1A</sub> autoreceptors may theoretically enhance serotonergic activity and contribute to antidepressant actions as well (Stahl, 2011; Hudziak, 2005; Pies, 1998). This partial agonist action also occurs at the level of the postsynaptic 5HT<sub>1A</sub> receptor, which may theoretically diminish some side effects shown by other SSRI drugs (Hudziak, 2005; Pies, 1998). Thanks

to this atypical combination of mechanisms of action, uncommon to other molecules such as trazodone or buspirone, VLZ has been theoretically deemed a better option (Dopheide, 2012), albeit this is yet to be scientifically demonstrated.

In veterinary medicine, trazodone (TRZ), another phenylpiperazine chemical derivative, has gained popularity as adjunctive off label medication for long term treatment of anxiety disorders in dogs (Gruen and Sherman, 2008) and as an anxiolytic for short-term management of patients after surgery (Jay et al., 2013; Chea and Giorgi, 2017). VLZ might have the potential to have better pharmacokinetic, pharmacodynamic and safety profiles than trazodone in the dog as theorized in human beings. However, to the best of Authors' knowledge, no studies concerning VLZ in dogs have been reported thus far in the literature. The present study aimed to evaluate the pharmacokinetics of a single oral 40 mg dose of VLZ in Labrador dogs in fasted and fed conditions.

## **MATERIALS AND METHODS**

### *Drugs and chemicals*

VLZ (Viibryd, 20 mg/tablet, Allergan, NJ, USA) for animal treatment was purchased at a regular pharmacy. VLZ HCl pure standard (purity 98%) was from Abcr GmbH, (Karlsruhe, Germany), while the internal standard trazodone (TRZ - IS) was from Sigma Chemical Co. (St. Louis MO, USA). Other reagents and chemicals of analytical grade were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Purified water was produced using the Milli-Q water purification system from Millipore, Inc. (Bedford, MA, USA).

### *Sample extraction procedure*

Dog plasma (300  $\mu$ L) and 30  $\mu$ L of TRZ - IS solution (500 ng/mL in methanol) were added to a 2.0 mL micro-centrifuge tube and vortexed for 20 s, followed by adding 30  $\mu$ L of methanol and 1.5 mL of dichloromethane. The mixture was then mixed for 5 min and left to separate into phases. The upper layer was removed and 1.2 mL was taken from the lower layer, transferred to a clean glass tube and evaporated to dryness under a stream of nitrogen at 40 °C. The residue was dissolved in 150  $\mu$ L of methanol and moved into LC vials.

#### *LC parameters*

The UHPLC/HPLC system Shimadzu Nexera X2 (Shimadzu, Japan) was used for VLZ LC analysis. Separation was performed on a Kinetex HILIC 100A (2.6 $\mu$ m, 50  $\times$  2.1 mm, Phenomenex, USA) with a KrudKatcher™ Ultra In-Line filter (Phenomenex, USA). The mobile phase consisted of acetonitrile (A) and 0.01M ammonium acetate, pH=7.0 (B). The following gradient elution program was applied: 98% A (0.0-1.3 min), 85% B (1.31-3.30 min) and 98% A (3.31-5.00 min). The flow rate of mobile phase was set at 0.8 mL/min and column temperature was kept at 30 °C. The injection volume was 5  $\mu$ L.

#### *MS parameters*

Mass spectrometry was performed using the QTRAP® 4500 triple quadrupole mass spectrometer (AB Sciex Framingham, USA). Detection was performed in positive electrospray ionization mode (ESI+) in the multiple-reaction monitoring mode (MRM), the following transitions were used: VLZ: m/z 442.2 > 197.0 and 442.2 > 246.0 and for TRZ - IS: m/z 372.2 > 176.0. The MS/MS parameters were optimised as follows: IonSpray voltage (IS) – 4500 V, Curtain Gas - 25 psi, Ion Source Gas 1 – 20 psi, Ion Source Gas 2 – 40 psi, gas temperature 450 °C. The Analyst 1.6.3 software controlled the UHPLC-MS/MS system and processed the data.

### *Validation procedure*

The developed method was fully validated in terms of linearity, precision (repeatability and within-laboratory reproducibility), recovery, limits of detection and quantification (LOD and LOQ). Linearity of the method (determination coefficient,  $R^2$ ) was validated by matrix-match calibration curves, which were prepared using canine blank plasma samples spiked at 8 different concentration levels (0.5, 1, 10, 25, 50, 100, 250 and 500 ng/mL) and was above 0.997. The repeatability was calculated after analysis of 6 plasma samples spiked with VLZ at 3 different concentrations (1, 10 and 50 ng/mL) on the same day with the same instrument and the same operator. For determination of reproducibility another two sets of 6 spiked samples were prepared as described above and analysed on two different days with the same instrument and different operators. The precision was calculated and expressed as the percentage coefficients of variation (CV, %) and was in the range of 4.3% to 8.4% and of 7.7% to 13.8% for repeatability and within-laboratory reproducibility, respectively. The extraction recovery experiment was carried out by analysing samples spiked at the same concentration levels as for the precision experiment. The mean extraction recovery of VLZ was  $92.10 \pm 3.56$  %. The LOD was estimated as the plasma concentration that produced a signal to noise ratio (S/N) of 3 and LOQ was determined as the lowest plasma concentration that produced a signal to noise ratio of 10. The LOD for VLZ was 0.1 ng/mL, the LOQ was 0.5 ng/mL.

### *Animals and experimental design*

The animal experiment was approved by the local welfare ethics committee and carried out in accordance with the European law (2010/63/UE). Six healthy, intact female (n=3) and male (n=3), adult (5–7 years) Labrador dogs with an average body weight of 35.5 kg (range 29.5–41.5 kg) were used. The dogs were determined to be clinically healthy based on physical examination and serum chemistry and

haematological analyses performed 48 hours before the study.

Animals were evaluated daily for visible adverse effects for 7 days following completion of the study by specialized personnel. Dogs were randomly assigned (blocked by sex to ensure a balanced distribution) to one of two treatment groups using software (Research Randomizer) and an open, single-dose, two-treatment, two-phase, cross-over study design. During the first phase, group I (n=3) was administered with VLZ HCl (Viibryd, 20 mg/tablet, Allergan, NJ, USA) at 40 mg/dog after fasting over-night. Group II (n=3) was fed prior to and after administration of the same dose. Canned dog food was provided as half the total amount 15 min before dosing, with the rest provided immediately after VLZ administration. On each study day, dogs were housed in individual cages and strictly monitored for potential coprophagia for 24 h. A two-week wash-out period was observed between the phases, then the treatment groups were reversed and the experiment was repeated. To facilitate blood sampling, 1 h before the commencement of the study, an 18 gauge soft cannula was inserted in the right medial saphenous vein. Blood samples (2 mL) were withdrawn at 5, 15, 30, 45 min and 1, 1.5, 2, 4, 6, 8, 10, 16, 24, 34, 48, 72 and 96 h after administration of VLZ. After centrifugation for 10 min at 1500 g, plasma was harvested, transferred in crio-vials and immediately frozen and stored at  $-20^{\circ}\text{C}$ .

#### *Pharmacokinetic analysis*

The concentration of VLZ vs time was pharmacokinetically analyzed using a non-compartment approach (ThothPro™ 4.3 software).  $C_{\max}$  was peak plasma concentration,  $T_{\max}$  was time at peak plasma concentration. The elimination half-life ( $t_{1/2\lambda z}$ ) was calculated using nonlinear least squares regression analysis of the concentration-time curve, and the areas under the curve (AUC) was calculated by the linear-up log-down rule to the final concentration-time point (Ct). From these values, the apparent volume of distribution ( $V_d = \text{dose} \times \text{AUMC}/\text{AUC}^2$ ), mean residence time ( $\text{MRT} = \text{AUMC}/\text{AUC}$ ) and

clearance ( $Cl = \text{dose}/AUC$ ) were determined. The relative bioavailability ( $F$ ) was calculated using the following equation:

$$(\%)F_{(\text{fasted})} = (AUC_{\text{fasted}}) / (AUC_{\text{fed}}) * 100$$

### *Statistical analysis*

Pharmacokinetic variables were evaluated using the student's  $t$ -test to determine statistically significant differences between groups. Pharmacokinetic parameters are presented as means  $\pm$  SD (normality tested by Shapiro-Wilk test).  $T_{\text{max}}$  (categorical variable) was expressed as median and range. Non-parametric test (Rank-Sum Test) was used to compare  $T_{\text{max}}$  values from two different treatments. Differences were considered significant if  $p < 0.05$ . All analyses were conducted using GraphPad InStat (GraphPad Software, La Jolla, CA, USA).

## **RESULTS**

The dose chosen in the present study was the same clinically used in humans, 40 mg. The drug was administered irrespective of the animal's body weight (2 intact 20 mg tablets) to avoid potential error in drug compounding. The dose, if normalized for the bodyweight, was different among the dogs (average 1.15 mg/kg, median 1.15 mg/kg, range 1.36-0.96 mg/kg).

VLZ concentrations were detectable in dogs' plasma in two different windows of time: 30 min to 10 h for the fasted group (one dog in this group had detectable concentration only up to 8h) and 4 h to 35 h for the fed group. The individual plasma concentration-time curves of VLZ are plotted in Fig. 1. The values of  $t_{1/2\lambda_z}$  were statistically different between the groups (fed,  $4.6 \pm 1.1$  h vs fasted,  $1.7 \pm 0.2$  h) in line with a different  $\lambda_z$  (fed,  $0.16 \pm 0.04$  1/h vs fasted,  $0.40 \pm 0.05$  1/h). Although the  $T_{\text{max}}$  drastically changed between the groups (fed, 10 h vs fasted, 1.5 h), the  $C_{\text{max}}$  did not significantly vary (fed,  $39.4 \pm$

5.6 ng/mL vs fasted,  $38.7 \pm 4.8$  ng/mL).  $C_{\max}$ , even if normalized for the dose expressed in mg/kg, was not statistically different between the two treatments (fed,  $34.5 \pm 3.6$  ng/mL vs fasted,  $34.3 \pm 6.8$  ng/mL). The AUC value was always statistically higher in the fed group. As a result, the average relative oral fasted bioavailability of VLZ was low,  $28.8 \pm 6.1\%$ . In the fed group AUC varied in a dose related fashion, while in the fasted group, the AUC individual values were quite steady (Fig. 3). The mean ( $\pm$ SD) pharmacokinetic parameters of VLZ are reported in Table 1. When  $C_{\max}$  and AUC values were plotted vs the administered dose in mg/kg, they appeared to vary less in unfed than in fed conditions. Indeed the slopes of the trend-lines in both parameters in the fed group are about 10 times higher than those computed in the fasted group (Fig. 4).

## DISCUSSION

VLZ is one of the latest FDA approved antidepressant drugs for humans. Theoretically, VLZ might be a suitable drug for use as a canine antidepressant, but no data have been reported to date on its pharmacokinetics, pharmacodynamics or safety profile in the dog. The present study was designed to characterize the pharmacokinetic features of 40 mg/dog VLZ after oral administration in fasted and fed dogs.

The recommended therapeutic dosage of VLZ for humans is 40 mg/person. In the present study the dose/dog was the same but as the dogs had a body weight half than human, the dose expressed in mg/kg was twice. This dose selection was based on the fact that no information about VLZ was present in dogs and several studies reported a lower oral bioavailability in canine if compared to humans (Muster et al., 2014).

The visual inspection of the individual pharmacokinetic curves showed that food can significantly affect the pharmacokinetic profile of VLZ. Mechanisms related to food effects on drug absorption have been

described under 5 categories: those causing decreased, delayed, increased or accelerated absorption, and those in which food has no significant effect (Welling, 1996). The findings of the present study showed that  $T_{max}$  and AUC were considerably increased in the fed group, feeding produced delayed and increased absorption.

The delayed absorption or decreased rate of absorption usually results from a slower gastric emptying rate and/or increased gastric pH resulting from the ingestion of food (Singh, 1999). The delayed absorption may also be expressed in terms of a larger MRT as reported in the present study. In fact, the slow rate of gastric emptying delays the onset of drug absorption, which usually occurs in the proximal region of the small intestine. The delayed drug absorption is likely to delay the onset of therapeutic action (Massarella et al., 1989; Spangler et al., 1987) which might not be clinically significant for a drug like VLZ that is administered over a long period.

The present study also reported an increased absorption in the fed group. Drugs that have an intestinal absorption that increases when they are administered with food include those that have an incomplete absorption as a consequence of their poor solubility in the GI fluids (Singh, 1999). VLZ is a water insoluble drug and is likely to belong to this category. The increased intestinal uptake may result from the delayed gastric emptying and increased secretion of bile salts which may increase the dissolution rate increasing the AUC values (Lennernas and Fager, 1997).

Surprisingly  $C_{max}$  value did not change between the groups. This circumstance has been previously reported in humans treated with ondansetron in fasted/fed states (Bozigian et al., 1994). Differently, administration of VLZ with food (high fat or light meal) increased  $C_{max}$  by approximately 147-160% in humans (Food and Drug Administration, 2015). Further studies are however needed to clarify this point. The difference in AUC values caused a relative oral fasted F% of about 30%, a bit lower than that reported in fasting humans (50%) (Schwartz et al., 2011; Boinpally et al., 2014). This is in agreement with other studies describing other drugs, reporting a lower oral F% in dogs when compared to humans

(Dohoo and Tasker, 1997; Giorgi et al., 2009; Giorgi et al., 2012a, 2012b; Muster et al., 2014).

VLZ has been shown to exhibit dose-proportional pharmacokinetics after single and multiple doses of 5 to 80 mg (Anonymous, 2012). In the human, a dose of 40 mg (a dose of 0.57 mg/kg assuming a patient with a body weight of 70 kg) produced an AUC of 1732 ng\*h/mL (Boinpally et al., 2014), while in the present study an average dose of 1.15 mg/kg produced an AUC of 487 ng\*h/mL. After normalization of the AUC for the dose, results indicated that the oral administration of the same dose produced an AUC in dogs that is around 14% of that in humans. If it is supposed that dogs and humans have the same mean effective plasma concentration, the canine effective dose should be around 7-folds higher than in humans. Pharmacokinetic/pharmacodynamics studies are needed to confirm this calculation.

The  $t_{1/2\lambda z}$  value of VLZ in fed dogs was longer than that in fasted dogs. Differences in elimination half-life are very difficult to evaluate because  $t_{1/2\lambda z}$  is a hybrid parameter that reflects not only elimination but also distribution (Toutain and Bousquet-Melou, 2004). Therefore, the origin of any differences among species in this parameter could be based on differences either in the elimination or distribution processes. Unfortunately, due to the water insolubility of VLZ, it was not possible to administer the drug IV, in order to calculate the absolute CL and Vd. However, the food itself might have trapped the drug releasing it slowly (reservoir effect) causing a flip flop effect (Toutain and Bousquet\_Melou, 2004). The  $t_{1/2\lambda z}$  reported in this study (4.6 h) was significantly shorter than that reported in humans (25 h) (Boinpally et al., 2014). Similar difference in  $t_{1/2\lambda z}$  between dogs and humans has been previously reported after trazodone oral administration (dogs, 2.7 h (Jay et al., 2013) vs humans 13 h (Kale and Agrawal, 2015)). These dissimilarities might be due to the diverse percentage of VLZ bound to plasma protein (96-99% in humans (Schwartz et al., 2011) but unknown in dogs). Indeed the high protein binding limits the unbound fraction of VLZ in the vascular system that can be presented to clearing organs. Another reason might be due to different metabolic rate or other elimination processes. VLZ is known to be metabolized in humans primarily by the cytochrome P-450 (CYP) 3A4 isozyme and non-CYP mediated (via

carboxylesterase [CES]) pathways (Boinpally, et al., 2014). It is known that dogs possess CYP 3A12/13 and VLZ might be a better substrate for these specific canine enzymes. CES1 and CES2 family enzymes share 40–50% amino acid sequence identity between dogs and man but have different substrate specificities (Sato et al., 2002; Imai et al., 2006). They catalyze the hydrolysis of a wide variety of substrates but there are few reports comparing the hydrolase activity of human organs with those of experimental animals (Yoshigae et al., 1998, Prueksaritanont et al., 1996). Also in this case canine CES1 and CES2 might metabolize VLZ quicker compared to humans'. Further specific studies are needed to clarify this point.

In summary, this is the first study concerning VLZ administration in dogs. After 40 mg/dog oral administration the plasma concentrations of VLZ were quantifiable. The unfed condition decreases the relative oral bioavailability to about 30%. Further studies are warranted to evaluate if VLZ can be as effective in dogs as it is in humans.

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## **CONFLICT OF INTEREST**

The authors declare that there are no conflicts of interest in publishing this work.

## **AUTHOR CONTRIBUTION**

M G and I S conceived the study design. B L-W, A L, M G and I S performed the experiments and collected samples. M G-S, A G and A P performed LC-MS/MS for drug analysis. All authors analyzed data. M G, A P and I S performed PK analysis. M G, C J K, A P wrote the draft paper and all the authors edited the manuscript and approved the final version.

## REFERENCES

- Anonymous, 2012. Forest Pharmaceuticals Inc. Viibryds (vilazodone hydrochloride) tablets: U.S. Prescribing Information. St. Louis, MO: Forest Pharmaceuticals, Inc.
- Boinpally, R., Gad, N., Gupta, S., Periclou, A., 2014. Influence of CYP3A4 induction/inhibition on the pharmacokinetics of vilazodone in healthy subjects. *Clin. Ther.* 36,1638-1649.
- Bozigian, H.P., Pritchard, J.F., Gooding, A.E., Pakes, G.E., 1994. Ondansetron absorption in adults: effect of dosage form, food, and antacids. *J. Pharm. Sci.* 83, 1011-1013.
- Chea, B., Giorgi, M., 2017. Trazodone: A review of its pharmacological properties and its off-label use in dogs and cats. *Am. J. Anim. Vet. Sci.* 12, 188-194.
- Crowell-Davis, S.L., Mattos de Souza Dantas, L., 2019. Introduction to Clinical Psychopharmacology for Veterinary Medicine, in: Crowell-Davis, S.L., Murray, T.F., Mattos de Souza Dantas, L. (Eds.), *Veterinary Psychopharmacology*. John Wiley & Sons Inc., pp. 53-65.
- Dohoo, S.E., Tasker, R.A., 1997. Pharmacokinetics of oral morphine sulfate in dogs: a comparison of sustained release and conventional formulations. *Can. J. Vet. Res.* 61, 251-255.
- Dopheide, J.A., 2012. Vilazodone's comparative merits yet to be demonstrated. *Am. J. Health. Syst. Pharm.* 69, 1549.
- Food and Drug Administration 2015. Viibryd (vilazodone hydrochloride): US prescribing information.
- Giorgi, M., Del Carlo, S., Saccomanni, G., Łebkowska-Wieruszewska, B., Kowalski, C.J.,

2009. Pharmacokinetic and urine profile of tramadol and its major metabolites following oral immediate release capsules administration in dogs. *Vet. Res. Commun.* 33, 875-885.

Giorgi, M., Meizler, A., Mills, P.C., 2012a. Pharmacokinetics of the novel atypical opioid tapentadol following oral and intravenous administration in dogs. *Vet. J.* 194, 309-313.

Giorgi, M., Portela, D.A., Breggi, G., Briganti, A., 2012b. Pharmacokinetics and pharmacodynamics of zolpidem after oral administration of a single dose in dogs. *Am. J. Vet. Res.* 73, 1650-1656.

Giorgi, M., Yun, H., 2012. Pharmacokinetics of mirtazapine and its main metabolites in Beagle dogs: a pilot study. *Vet. J.* 192, 239-241.

Gruen, M.E., Sherman, B.L., 2008. Use of trazodone as an adjunctive agent in the treatment of canine anxiety disorders: 56 cases (1995–2007). *J. Am. Vet. Med. Assoc.* 233, 1902–1907.

Hudziak, J., Waterman, C.S., 2005. Buspirone, in: Sadock, B.J., Sadock, V.A. (Eds.), Kaplan and Sadock's comprehensive textbook of psychiatry (8th edn), Lipincott Williams & Wilkins: Philadelphia, New York.

Imai, T., Taketani, M., Shii, M., Hosokawa, M., Chiba, K., 2006. Substrate specificity of carboxylesterase isozymes and their contribution to hydrolase activity in the human liver and small intestine. *Drug. Metab. Dispos.* 34, 1734–1741.

Jay, A.R., Krotscheck, U., Parsley, E., Benson, L., Kravitz, A., Mulligan, A., Silva, J., Mohammed, H., Schwark, W.S., 2013. Pharmacokinetics, bioavailability, and hemodynamic effects of trazodone after intravenous and oral administration of a single dose to dogs. *Am. J. Vet. Res.* 74, 1450-1456.

Kale, P., Agrawal, Y. K., 2015. Pharmacokinetics of single oral dose trazodone: a randomized, two-period, cross-over trial in healthy, adult, human volunteers under fed condition. *Front. Pharmacol.* 6, 224.

Lavy, E., Prise, U., Soldani, G., Neri, D., Brandriss, N., Bar Chaim, A., Giorgi, M., 2011. Pharmacokinetics of methylphenidate after oral administration of immediate and sustained-release preparations in Beagle dogs. *Vet. J.* 189, 336-340.

Lennernas, H., Fager, G., 1997. Pharmacodynamics and pharmacokinetics of the HMG-CoA reductase inhibitors. *Clin. Pharmacokinet.* 32, 403-425.

Musther, H., Olivares-Morales, A., Hatley, O.J.D., Liu, B., Rostami Hodjegana, A., 2014. Animal versus human oral drug bioavailability: Do they correlate? *Eur. J. Pharm. Sci.* 57, 280-291.

Massarella, J.W., DeFeo, T.M., Brown, A.N., Lin, A., Wills R. J., 1989. The influence of food on the pharmacokinetics and ACE inhibition of cilazapril. *Br. J. Clin. Pharmacol.* 27, 205-209.

Overall, K.L., 2013. *Manual of clinical behavioral medicine for dogs and cats* Elsevier, St. Louis, MO.

Pies, R., 1998. *Handbook of Essential Psychopharmacology*, American Psychiatric Press Inc: Washington, DC.

Prueksaritanont, T., Gorham, L.M., Hochman, J.H., Tran, L.O., Vyas, K.P., 1996. Comparison studies of drug-metabolizing enzymes in dog, monkey, and human small intestine, and in caco-2 cells. *Drug. Metab. Dispos.* 24, 634-642.

Satoh, T., Taylor, P., Bosron, W.F., Sanghani, P., Hosokawa, M., Du, B.N., 2002. Current progress on esterases: from molecular structure to function. *Drug. Metab. Dispos.* 30, 488-493.

Schwartz, T.L., Siddiqui, U.A., Stahl, S.M. Vilazodone: a brief pharmacological and clinical review of the novel serotonin partial agonist and reuptake inhibitor. *Ther. Adv. Psychopharmacol.* 1, 81-87.

Singh, B.N., 1999. Effects of Food on Clinical Pharmacokinetics. *Clin. Pharmacokinet.* 37, 213-255.

Spangler, D.L., Lutz, C.N., Tinkelman, D., 1987. The effect of diet on the efficacy onset and

duration of action of procaterol hydrochloride tablets. *Ann. Allergy.* 58, 59-62.

Stahl, S., 2011. *Essential Psychopharmacology: the Prescriber's Guide*, 4th edn, Cambridge University Press: Cambridge.

Toutain, P.L., Bousquet-Melou, A., 2004. Plasma terminal half-life. *J. Vet. Pharmacol. Ther.* 27, 427-439.

Welling, P.G., 1996. Effects of food on drug absorption. *Annu. Rev. Nutr.* 16, 383-415.

Yoshigae, Y., Imai, T., Horita, A., Matsukane, H., Otagiri, M., 1998. Species differences in stereoselective hydrolase activity in intestinal mucosa. *Pharm. Res.* 15, 626-631.

## Table

**Table 1.** Mean  $\pm$  SD value of the pharmacokinetic parameters of VLZ following a single oral administration at a dosage of 40 mg/subject in fasted and fed dogs ( $n = 6$ /group).

Pharmacokinetic parameter (unit)		VLZ					
		Fed			Fasted		
$\lambda_z$	(1/h)	0.16	$\pm$	0.04	0.40*	$\pm$	0.05
$t_{1/2\lambda_z}$	(h)	4.64	$\pm$	1.07	1.73*	$\pm$	0.19
$C_{max}$	(ng/mL)	39.44	$\pm$	5.59	38.68	$\pm$	4.76
$T_{max\ddagger}$	(h)	10	$\pm$	10-10	1.5*	$\pm$	1.5-1.5
CL/F	(L/g*h)	0.0025	$\pm$	0.0005	0.0088*	$\pm$	0.0015
V/F	(L/g)	0.0163	$\pm$	0.0010	0.0223*	$\pm$	0.0043
$AUC_{0-last}$	( $\mu\text{g}\cdot\text{h/L}$ )	471.98	$\pm$	106.62	128.94*	$\pm$	11.09
$AUC_{0-\infty}$	( $\mu\text{g}\cdot\text{h/L}$ )	486.98	$\pm$	115.65	134.63*	$\pm$	12.37
$MRT_{0-\infty}$	(h)	15.52	$\pm$	1.28	3.91*	$\pm$	0.43
F%					28.80	$\pm$	6.12

Note:  $\lambda_z$  = terminal phase rate constant,  $t_{1/2\lambda_z}$  = terminal half-life,  $C_{max}$  = peak plasma concentration,  $T_{max}$  = time of peak concentration, CL/F = plasma clearance normalized for F, Vd = volume of distribution normalized for F,  $AUC_{0-last}$  = area under the curve from 0 to last time collected samples,  $AUC_{0-\infty}$  = area under the curve from 0 h to infinity,  $MRT_{0-\infty}$  = mean residence time, F = relative bioavailability,  $\ddagger$ Median value and range, \*  $p < 0.05$

Figures' caption

Figure 1. Dual mechanism of action of VLZ.

Figure 2. Individual plasma VLZ concentration following single oral administration of 40 mg/dog Viibryd in fasted (--○--, n=6) and fed (—●—, n=6) dogs.

Figure 3. Correlation between AUC individual values in the fed (●) and fasted (△) group in dogs (n=6)

Figure 4. Coefficient of determination ( $R^2$ ) and estimated intercept and slope between individual weight-adjusted vilazodone doses and AUC (upper panel) or  $C_{\max}$  (lower panel), in fasted (--○--, n=6) and fed (—●—, n=6) dogs.

Figure 1

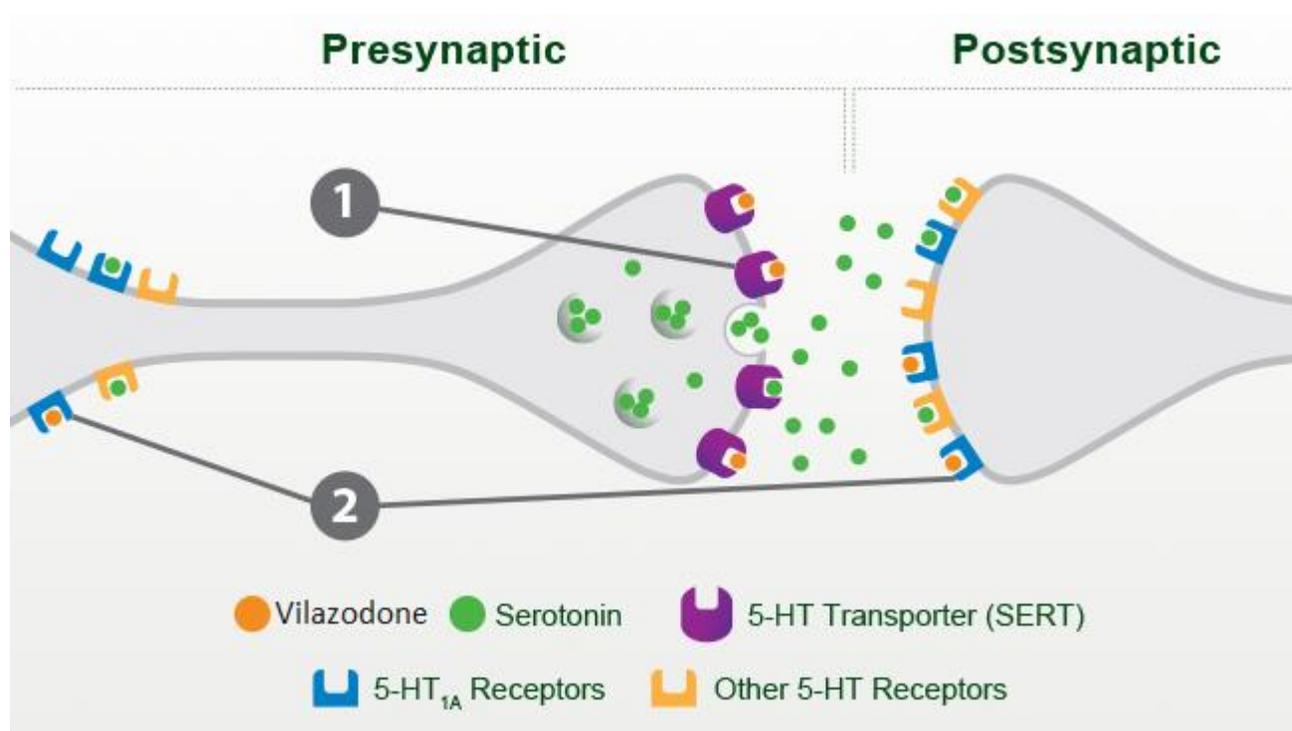
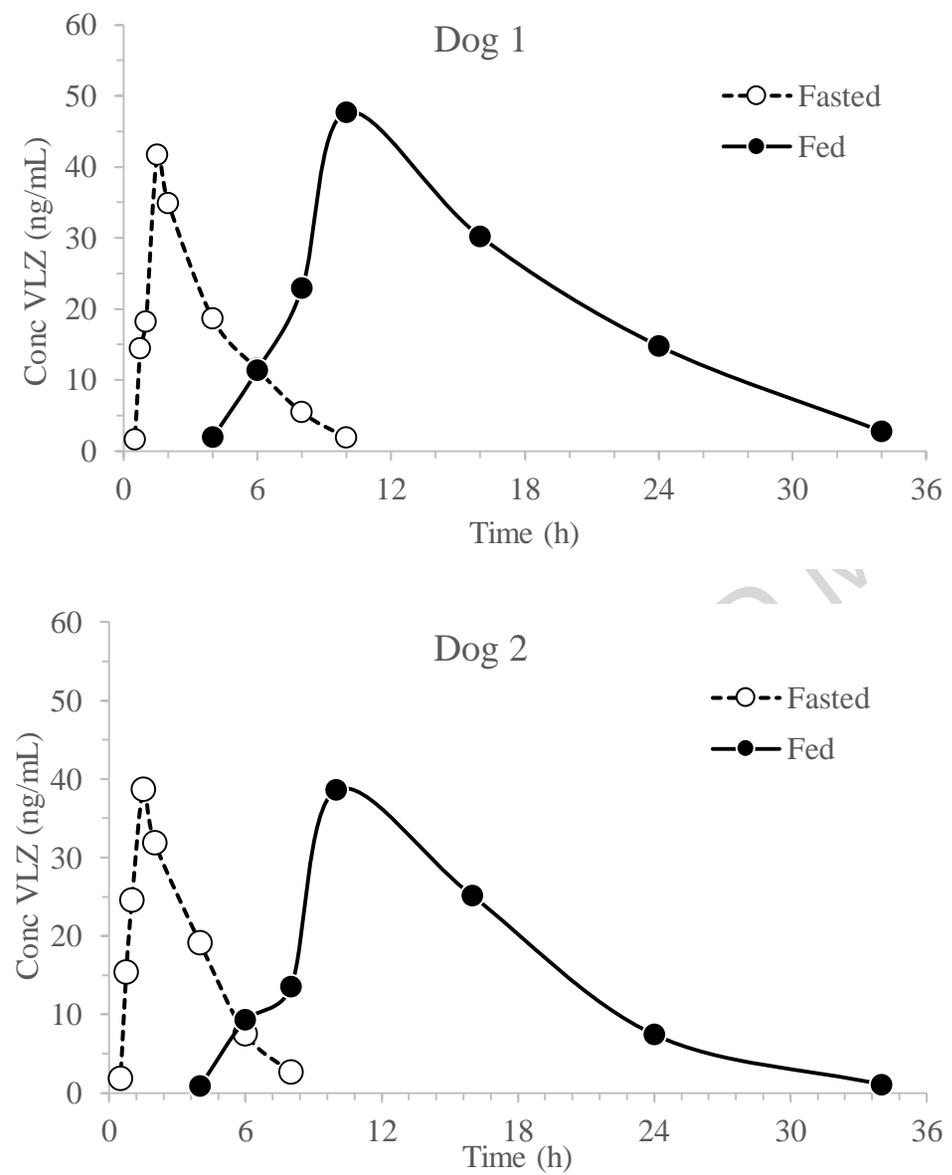
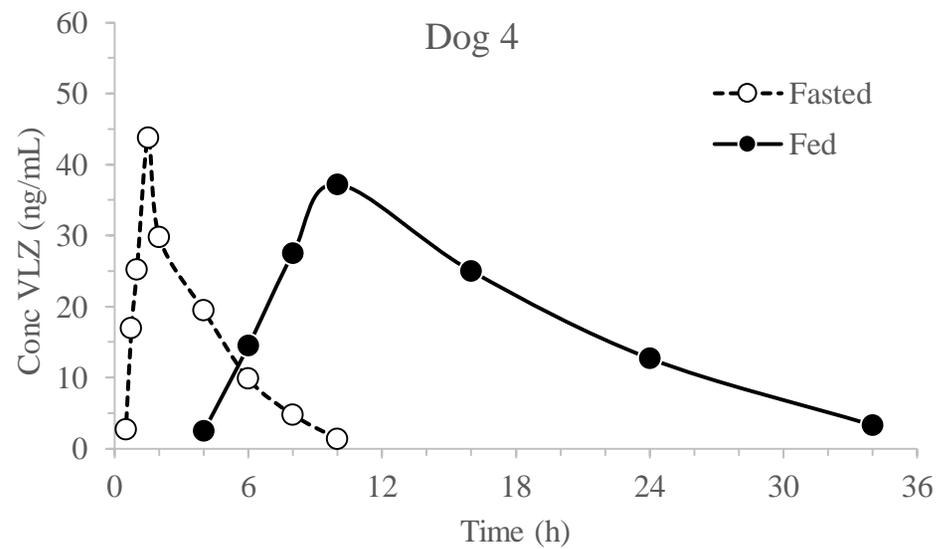
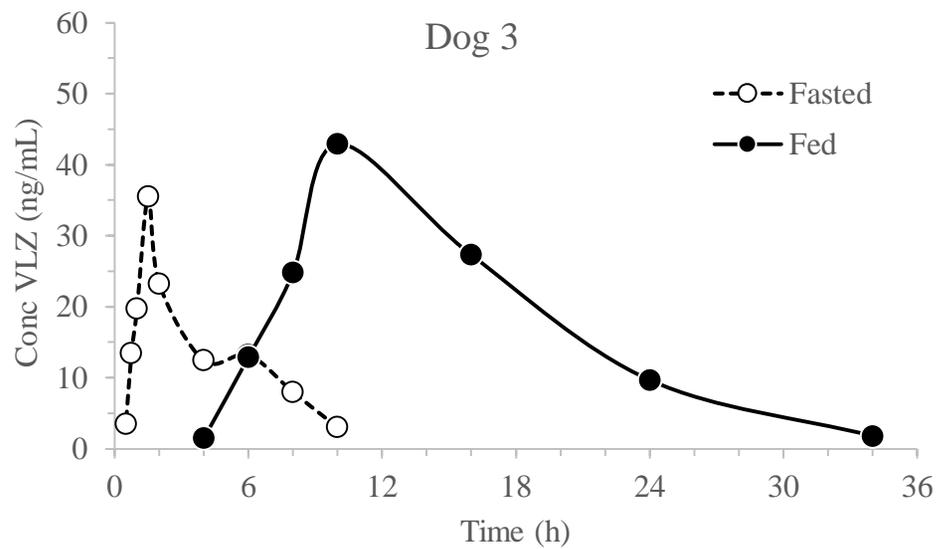


Figure 2





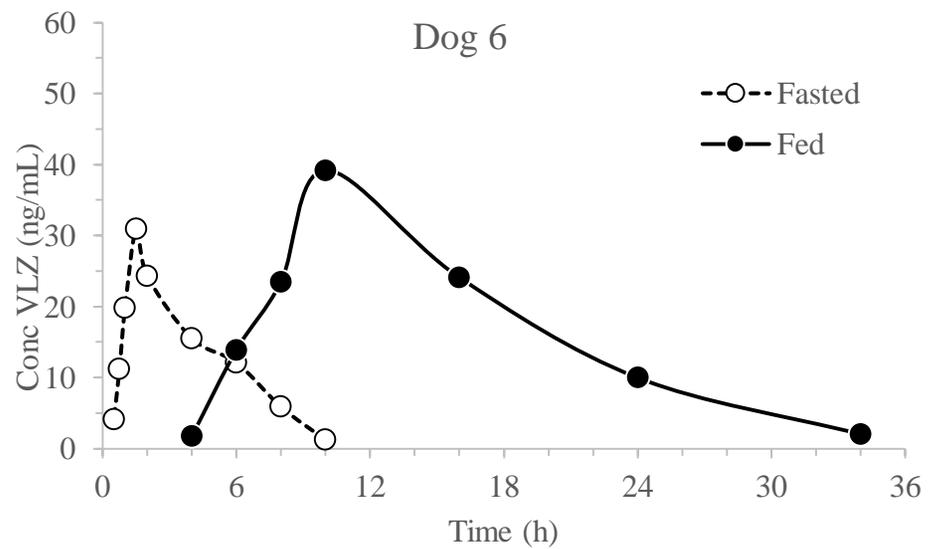
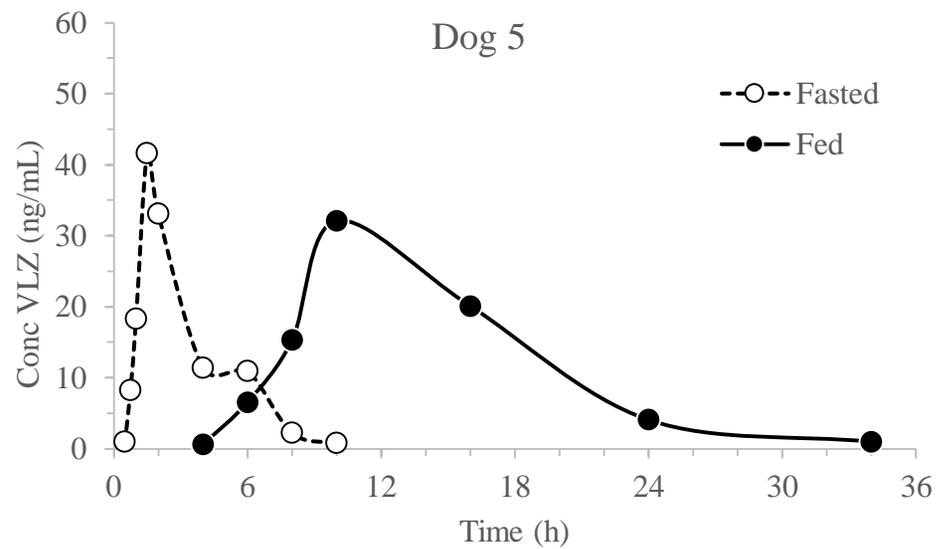


Figure 3

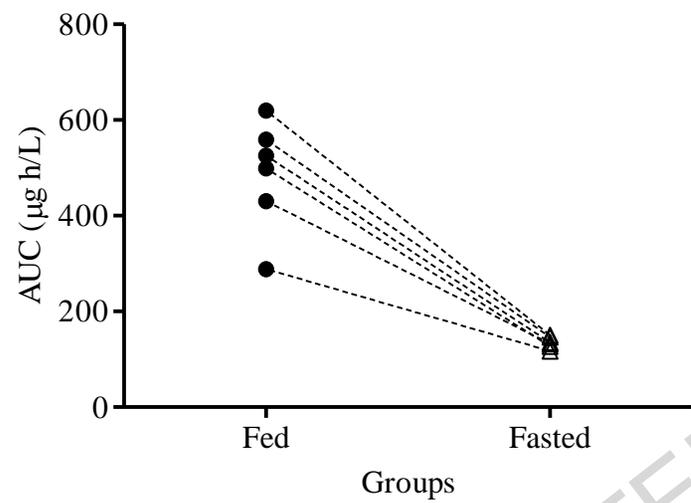
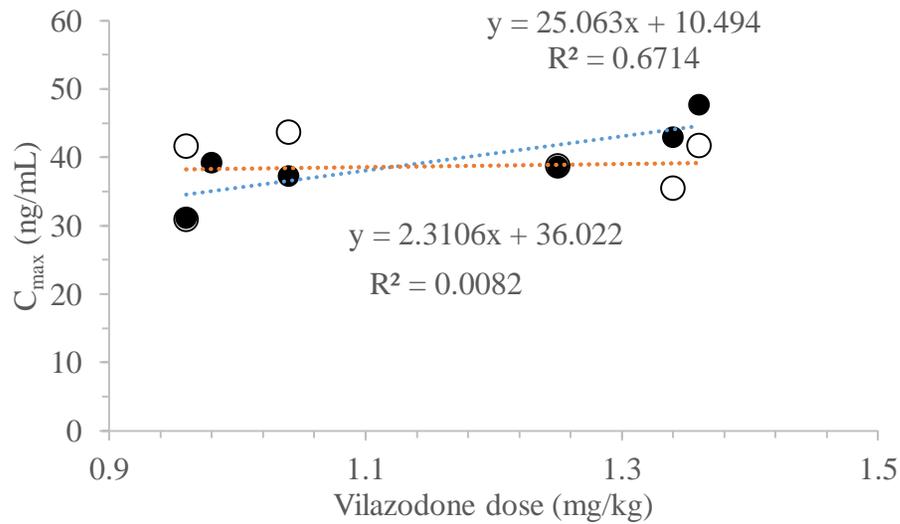
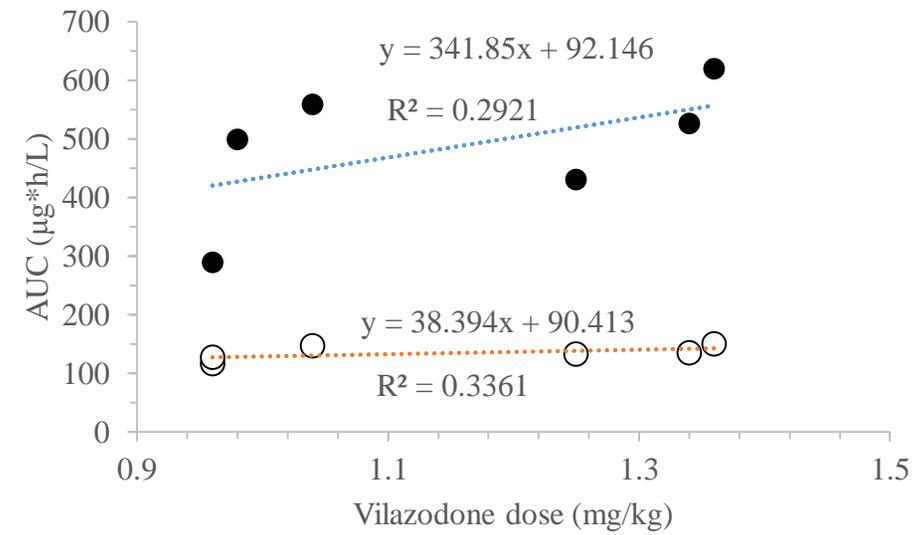


Figure 4



ACCEPTED MANUSCRIPT

- The fed status increased  $T_{\max}$  and AUC but did not change  $C_{\max}$  of VLZ in dogs
- The relative oral bioavailability of VLZ in fasted dogs was low (28.8%)
- The oral administration of the human dose produced an AUC in dogs that is around 14% of that in humans

ACCEPTED MANUSCRIPT

Presynaptic

Postsynaptic

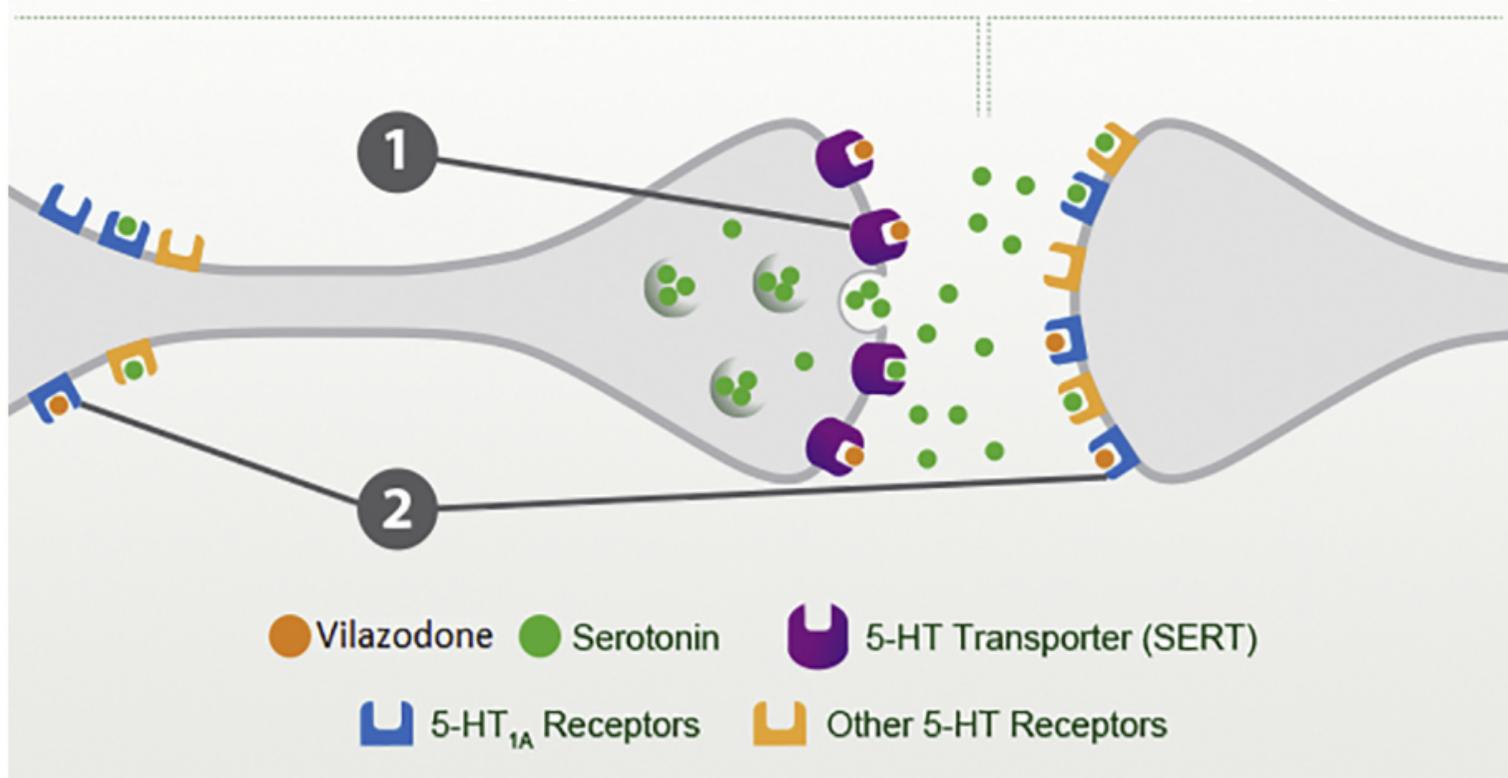


Figure 1

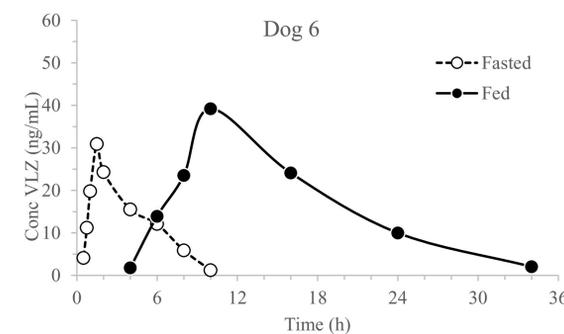
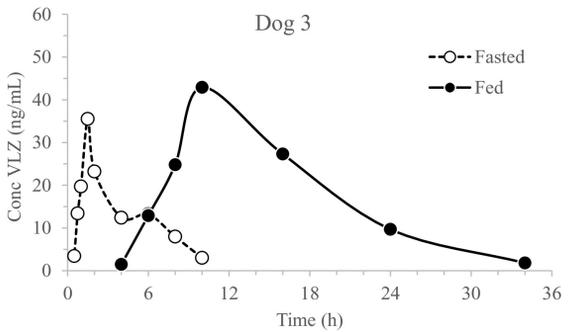
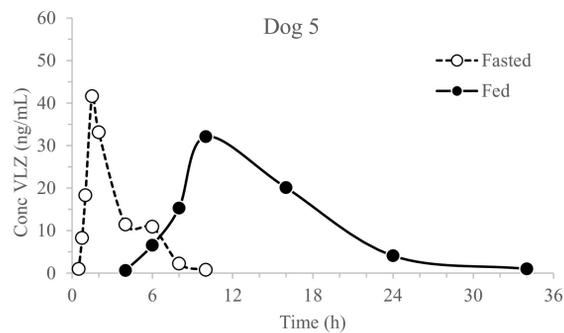
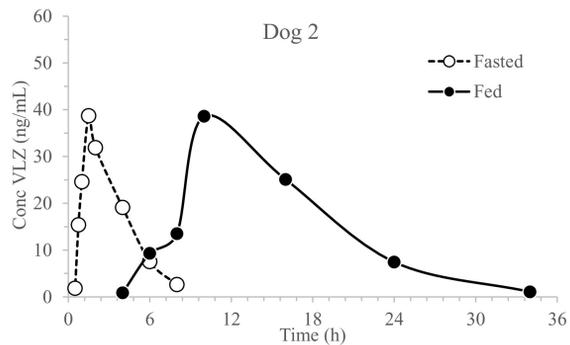
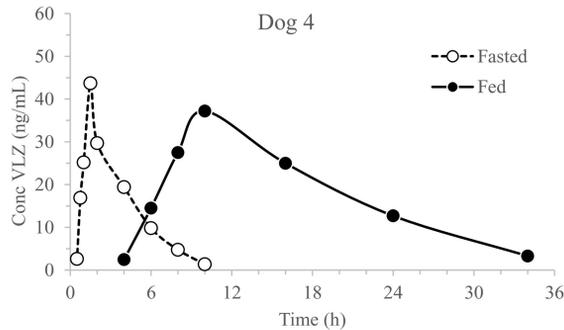
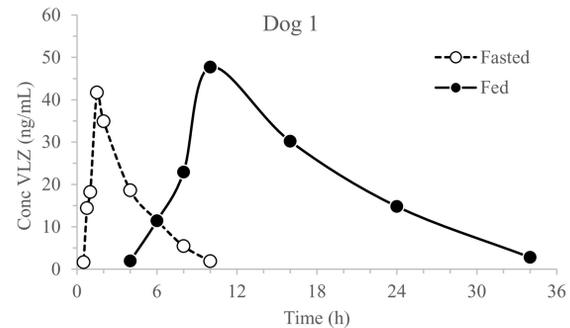


Figure 2

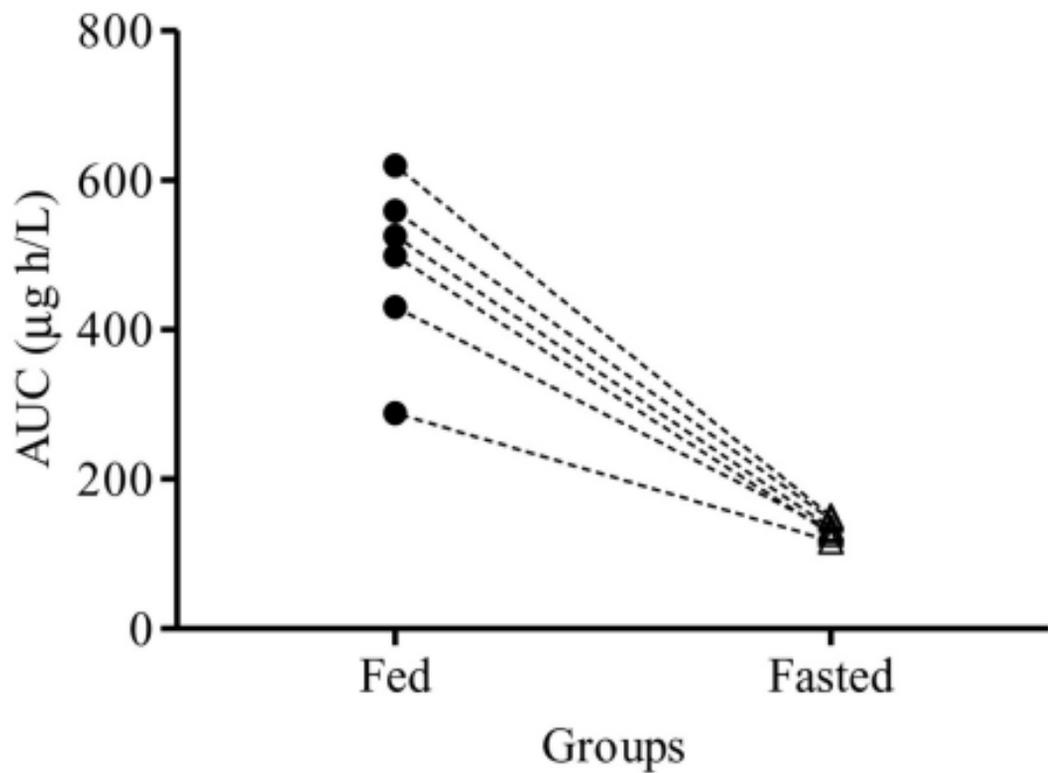


Figure 3

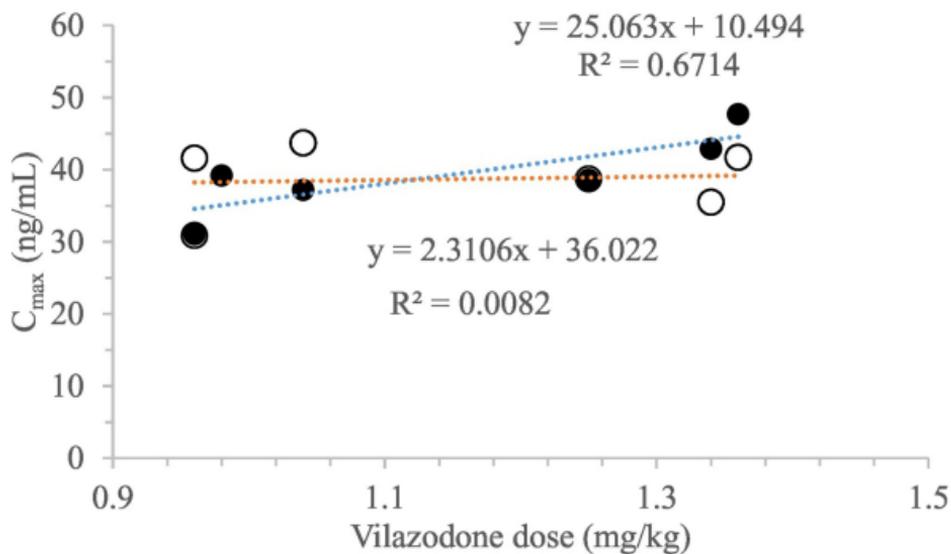
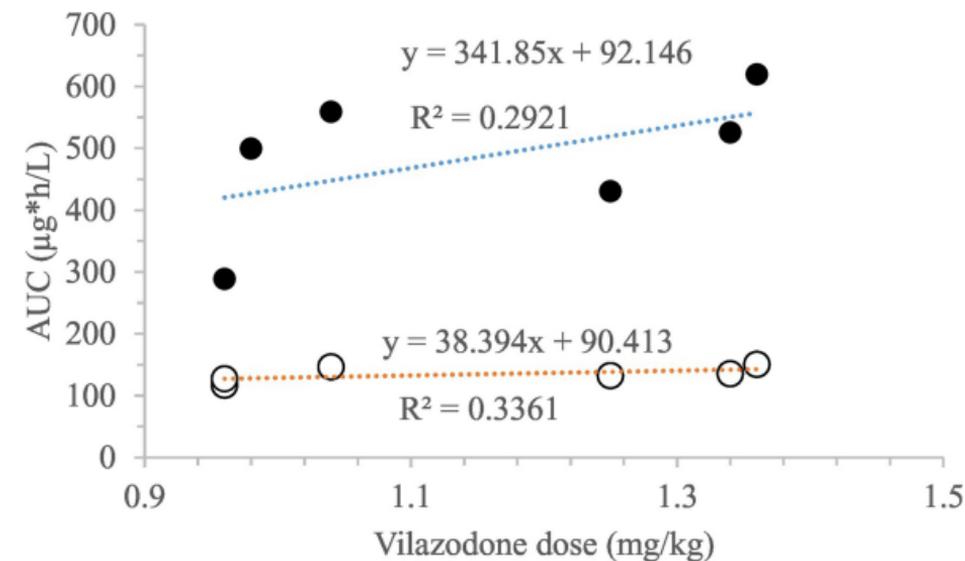


Figure 4