

## Pharmacokinetics and Lung Tissue Concentration of Valnemulin in Swine

Z. Zhang, C.Y. Zhang, J.P. Guo, L.X. Zhu, X.Y. Luo, R. Wang and Y.H. Liu

Laboratory of Veterinary Pharmacology, College of Veterinary Medicine,  
South China Agricultural University, 483 Wu Shan Street, Tian He District, Guangzhou, China

**Abstract:** The systemic bioavailability and lung tissue distribution of valnemulin were investigated in swine. About 65 pigs received 10 mg kg<sup>-1</sup> body weight of valnemulin by either intravenous (i.v.) or oral (p.o.) route in two studies: study A (10 pigs, i.v. or p.o.) and study B (55 pigs, p.o.). The plasma and lung tissue concentration of the drug were determined by a validated HPLC-MS/MS method. Plasma concentration-time data after i.v. administration (10 mg kg<sup>-1</sup> b.w.) were best described by a two-compartment open model. The pharmacokinetic parameters were elimination rate ( $k_e$ ) 0.95±0.17 h<sup>-1</sup>, the maximum concentrations 4.63±0.66 µg mL<sup>-1</sup>, area under the plasma concentration-time curve (AUC<sub>inf</sub>) 5.30±0.37 (h\*µg)mL<sup>-1</sup>. On the other hand, A one-compartment model with a 1st order absorption rate was best fitted to the plasma concentration-time curve of valnemulin after oral administration (10 mg kg<sup>-1</sup> b.w.) and the absorption rate ( $k_a$ ) was 0.34±0.03 h<sup>-1</sup>, the elimination rate ( $k_e$ ) was 1.05±0.19 h<sup>-1</sup>, the maximum concentration was 0.59±0.08 µg mL<sup>-1</sup> at 1.98±0.21 h (t<sub>max</sub>), the mean p.o. bioavailability (F) was 57.43%. Following p.o. administration, a mean valnemulin concentration of 0.14 µg g<sup>-1</sup> was detected in lung tissue at 36 h postdosing. The lung AUC<sub>inf</sub> (410.16 h\*µg g<sup>-1</sup>) was 77.39 times higher than the corresponding plasma AUC<sub>inf</sub> (5.30 h\*µg g<sup>-1</sup>). The apparent elimination half-time for valnemulin in lung was 3.57 h. The advisable bioavailability and extensive distribution to lung tissue following a single dose of valnemulin may be desirable pharmacokinetic attributes for an antimicrobial drug used for the treatment and prevention of respiratory disease in swine.

**Key words:** Valnemulin, pharmacokinetic, pig, bioavailability, HPLC-MS/MS, China

### INTRODUCTION

Valnemulin is produced semi-synthetically from pleuromutilin (Birch *et al.*, 1996) which also includes tiamulin, retapamulin (Gentry *et al.*, 2008; Hu and Zou, 2009), a product of fermentation from the Basidiomycete *Pleurotus mutilis*. The antibacterial action of valnemulin is inhibition of protein synthesis through binding to the 50s ribosomal subunit of bacteria and preventing RNA synthesis at higher levels.

Valnemulin is used in the swine industry to treat infections with swine dysentery, porcine proliferative enteropathy and for the treatment and prevention of swine enzootic pneumonia. Mycoplasma is a very important disease of pigs that causes severe economic loss through clinical disease and an adverse effect on food conversion and weight gain.

Some researchers have reported that valnemulin appeared to have exceptional activity against *M. hyopneumoniae* (Hannan and Ripley, 1996) with promise that it will be more effective in treatment of enzootic pneumonia than tiamulin (Ross, 1999). With the intensity of valnemulin use in the treatment of swine

dysentery in the pig industry, the field reports of reduced clinical efficiency of valnemulin in the treatment of swine dysentery was increasingly (Lobova *et al.*, 2004; Hidalgo *et al.*, 2009) highlighted the need for detailed information of valnemulin pharmacokinetic properties. However to the researchers' knowledge, there was a little detailed information on valnemulin pharmacokinetic behavior in pigs (Horkovics-Kovats and Schatz, 1996, 1997). The present studies were undertaken to determine the bioavailability and lung pharmacokinetics of valnemulin in swine following i.v. and p.o. administration.

### MATERIALS AND METHODS

**Drugs and reagents:** Valnemulin (standard, 98.7%) was purchased from J and K chemical Ltd. (Beijing, China). Valnemulin hydrochloride (89%) was provided by Guang dong Da hua nong Animal Health Products Co., Ltd. (Guangdong, China). Methanol and acetonitrile of HPLC grade were obtained from Fisher Scientific (Pittsburg, USA). All other chemicals (analytical grade) were purchased from Sigma-aldrich.

**Corresponding Author:** Ya Hong Liu, Laboratory of Veterinary Pharmacology, College of Veterinary Medicine,  
South China Agricultural University, 483 Wu Shan Street, Tian He District, Guangzhou, China

**Animals:** Ten healthy male Duroc x (Landrace x Yorkshire) cross pigs (about 6 weeks; weight range 7-10 kg) were used in study A.

About 55 healthy male Duroc x (Landrace x Yorkshire) cross pigs (about 4 months; weight range 64-80 kg) were used in study B.

The animals were provided with a nonmedicated commercial concentrate diet and tap water *ad libitum*. The studies were designed and conducted in accordance with Institutional Animal Care and Use Committee (IACUC) protocols in South China Agricultural University.

**Experimental procedure:** The pharmacokinetic and lung tissue concentrations of valnemulin were investigated in two studies. The studies are listed as follows; study A is on pharmacokinetics and oral bioavailability of valnemulin in swine and study B is on lung distribution of valnemulin in swine.

**Study A: Pharmacokinetics and bioavailability following p.o. administration:** The study was designed as a two-way-crossover study including i.v. administration, p.o. administration. One week was elapsed between experiment. Intravenous injections were made via a sterile catheter into an ear vein while oral administration was made into stomach with a stomach tube.

The dosage was a single valnemulin hydrochloride (sterile powder) 10 mg (valnemulin equivalents)  $\text{kg}^{-1}$  b.w. Blood samples ( $1.5 \text{ mL sample}^{-1}$ ) were collected serially from the anterior vena cava at 0.167, 0.25, 0.5, 0.75, 1, 2, 3, 4, 6, 8, 10, 12, 16 h after i.v. and p.o. administration and then treated immediately with heparin and centrifuged at 1500 g for 10 min to separate the plasma which was stored at  $-20^\circ\text{C}$  until analysis; samples were assayed within 15 days of collection. The amount of blood collected during the sampling period never exceeded 10% of the total body blood.

**Study B: Lung distribution:** About 55 pigs were divided into 11 groups. A single dose of valnemulin ( $10 \text{ mg kg}^{-1}$  b.w.) was orally administered. Animals were killed by captive bolt stunning followed by pithing and exsanguination. Blood and lung tissue samples were taken at 0.25, 0.5, 0.75, 1, 2, 4, 8, 12, 24 and 36 h after administration. The blood samples were transferred into heparinized tubes and immediately centrifuged at 1500 g for 10 min and plasma samples were stored at  $-20^\circ\text{C}$  until analysis. Lung tissue samples were frozen at  $-20^\circ\text{C}$  until analysis after cleaning the bloodstain with 0.9% NaCl. All samples were assayed within 15 days of collection.

#### **Valnemulin assay**

**Instrumentation and chromatographic conditions:** Plasma samples were carried out on an Agilent 1200 series high performance liquid chromatography (Agilent, CA, USA). Chromatographic separations were performed using a  $5.0 \mu\text{m}$  pore size phenomenex Luna C18 column ( $150 \times 2.1 \text{ mm I.D.}$ ). The column temperature was  $35^\circ\text{C}$ . A mobile phase was consisted of acetonitrile: 0.1% formic acid containing 2 mM ammonium acetate (35:65, v/v) delivered at a flow rate of  $0.2 \text{ mL min}^{-1}$ . The injection volume was  $5 \mu\text{L}$ .

**Mass spectrometric conditions:** Analyses were performed using API 4000 triple quadrupole TSQ quantum mass spectrometer (Applied Biosystems Corporation, CA, USA). Mass spectrometry was performed using Biomed equipped with an ESI source. The specific precursor-to-ion transitions monitored were  $m/z$  565.0 $\rightarrow$ 263.2 and 565.0 $\rightarrow$ 285.3. Spray voltage was optimized at 4000 V, source temperature at  $500^\circ\text{C}$ , curtain gas and auxiliary gas (nitrogen) pressure at 25 and 10 psi, respectively. Nitrogen was used as collision gas at a pressure of 4.0 psi and collision energy was set at 24 and 32 V and collision extraction potential at 15 and 12 V for transition  $m/z$  565.0 $\rightarrow$ 263.2 and 565.0 $\rightarrow$ 285.3, respectively. The dwell time used was 300 m sec.

**Sample extraction:** Plasma samples were thawed and centrifuged to remove particulates. An aliquot of 0.5 mL plasma was deproteinized with 0.5 mL acetonitrile and then vigorously vortexed for 1 min followed by centrifugation (10000 g for 10 min). The upper supernatant layer was filtered through a  $0.22 \mu\text{m}$  nylon filter into an autosampler vial.

Each of homogenized (10000 g, 1 min) lung tissue samples (2 g each) was accurately weighed in a plastic tube and 8 mL acetonitrile was added. Samples were then shaken (30 min) and centrifuged (6000 g, 10 min). The supernatant was filtered through a Solid Phase Extraction (SPE) column. The elute was evaporated to dryness under nitrogen ( $40^\circ\text{C}$ ) and the sample was reconstituted in 2 mL acetonitrile for analysis.

Standard calibration curves of plasma and lung tissue were considered linear from  $0.01\text{-}2 \mu\text{g mL}^{-1}$  to  $0.01\text{-}10 \mu\text{g g}^{-1}$ , respectively ( $r^2 > 0.99$ ). The limit of quantitation was  $0.01 \mu\text{g mL}^{-1}$  (or  $0.01 \mu\text{g g}^{-1}$ ) for both plasma and lung tissue samples. The interday and intraday coefficients of variation were  $< 10\%$  and recoveries ranged from 90.03- 96.25%, respectively.

**Data analysis:** Pharmacokinetic analysis was performed using WinNonlin professional (Version 5.0.1, Pharsight) software. The pharmacokinetic parameters of study A

were used for the compartmental analysis. On the other hand, the time and concentration data of study B were applied to the noncompartmental analysis. Compartmental analysis was evaluated based on coefficient of determination and Akaike's Information Criteria (AIC) for the best fit model (Yamaoka *et al.*, 1978; Riviere, 1999). Area under the plasma concentration-time curve (AUC) was computed using the trapezoidal method with the last triangle extrapolated to infinity (Buur, 2007).

Area under the plasma concentration-time curve (AUC) was computed using the linear up/log down method which meant that the linear trapezoidal method in applied for increasing or equal concentrations and the log-linear trapezoidal method for decreasing concentrations (Gabrielsson and Weiner, 1997).

AUC<sub>extra</sub> (AUC extrapolated from time last to infinity) should ideally be as small as possible in comparison to the total area and did not exceed 20% of AUC<sub>total</sub> (AUC extrapolated from 0 to ∞) (Gabrielsson and Weiner, 1997). Pharmacokinetic parameters were analysed using a linear mixed model with a two-way Analysis of Variance (ANOVA) to examine the difference between periods. Because population AUC values tend to deviate from normality, AUC comparisons are conducted on log transformed estimates (Martinez, 1998). The 5% level of significance examine (p<0.05) was used thought for statistical hypothesis tests (Toutain and Bousquet-Melou, 2004; Hauschke *et al.*, 2007).

**RESULTS AND DISCUSSION**

The method used in this study was selective and stable and the good linear range of valnemulin in pig plasma and lung tissue were 0.01-2 µg mL<sup>-1</sup> and 0.01-10 µg g<sup>-1</sup>, respectively (r<sup>2</sup>>0.99). No interferences from endogenous components or other sources were found. The Limits of Quantitation (LOQ) was 10 ng mL<sup>-1</sup> (or 10 ng g<sup>-1</sup>) for both plasma and lung tissue samples. Valnemulin was a well-tolerated drug in pigs. All animals remained in good health throughout the acclimatization and study periods. No side effects were observed after a single i.v. and p.o. administration (10 mg kg<sup>-1</sup> b.w.).

**Study A: Pharmacokinetics and oral bioavailability:** Data from each individual animal were processed separately and pharmacokinetic curve and parameters were calculated. Plots of the mean valnemulin concentration in plasma after the single i.v. and p.o. administration using 10 mg kg<sup>-1</sup> b.w. valnemulin were shown in Fig. 1. The pharmacokinetic parameters of the aforementioned doses and application routes were shown in Table 1.

**Study B: Lung distribution:** In lung tissue, average valnemulin concentrations in lung and plasma at various

Table 1: The pharmacokinetic parameters of valnemulin (mean±SE, n = 10) after intravenous and oral administration of 10 mg kg<sup>-1</sup> b.w. in pigs (study A)

Parameters	Units	(Mean±SE)	
		i.v.	p.o.
k <sub>a</sub>	h <sup>-1</sup>	-	0.34±0.03
k <sub>e</sub>	h <sup>-1</sup>	0.95±0.17	1.05±0.19
t <sub>lag</sub>	h	-	0.16±0.03
α	h <sup>-1</sup>	6.03±1.15	-
β	h <sup>-1</sup>	0.36±0.03	-
V <sub>ss</sub>	L kg <sup>-1</sup>	5.28±0.35	-
V <sub>F</sub>	L kg <sup>-1</sup>	-	10.79±1.49
AUC <sub>inf</sub>	(h*µg) mL <sup>-1</sup>	5.30±0.37*	3.12±0.39
AUC <sub>extra</sub>	%	2.56±0.46	2.82±0.31
CL	L/h/kg	2.03±0.14	-
CL <sub>F</sub>	L/h/kg	-	3.51±0.42
C <sub>max</sub>	µg mL <sup>-1</sup>	4.63±0.66	0.59±0.08
t <sub>max</sub>	h	-	1.98±0.21
t <sub>1/2ka</sub>	h	-	2.20±0.19
t <sub>1/2ke</sub>	h	0.92±0.46	0.92±0.21
F	%	-	57.43±3.89

Significant differences (\*p<0.05). k<sub>a</sub> = The absorption rate. k<sub>e</sub> = The elimination rate. t<sub>lag</sub> = The lag time. F = the systemic bioavailability. V<sub>ss</sub> = An estimate of the volume of distribution at steady state. V<sub>F</sub> = The apparent volume of distribution (where, F is the fraction of dose absorbed). AUC<sub>inf</sub> = AUC from time zero extrapolated to infinity. CL = The total body clearance. CL<sub>F</sub> = The total body clearance (where, F is the fraction of dose absorbed). C<sub>max</sub> = The maximum concentration. t<sub>lag</sub> = The lag time. t<sub>max</sub> = The time to maximum concentration. t<sub>1/2ka</sub> = The absorption half-lives. t<sub>1/2ke</sub> = The elimination half-lives. α = The macro rate constant associated with The distribution phase. β = The macro rate constant associated with the elimination phase. AUC<sub>extra</sub> = Percentage of AUC<sub>inf</sub> due to extrapolation from t<sub>last</sub> to infinity

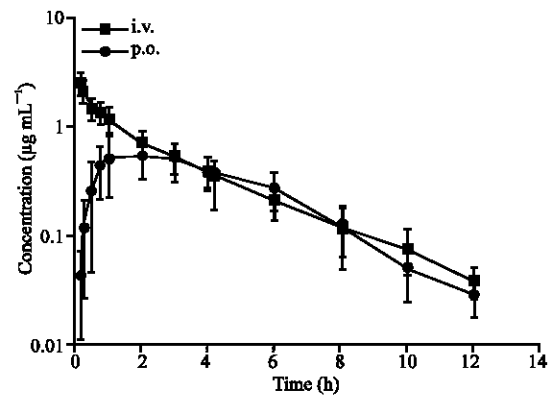


Fig. 1: Semilogarithmic graph depicting the time-plasma concentration course of valnemulin in pigs after intravenous and oral administration of 10 mg kg<sup>-1</sup> b.w. (error bars indicate standard deviation) of valnemulin

time points were showed in Fig. 2. A mean valnemulin concentration of 0.14 µg g<sup>-1</sup> was detected by 36 h following p.o. administration. The highest mean lung concentration (C<sub>max</sub>) was 49.86 µg g<sup>-1</sup>, reached 4 h after oral administration (t<sub>max</sub>). The lung:plasma ratio of AUC values were shown in Table 2. The lung AUC<sub>inf</sub> was 77.39 times greater than the corresponding plasma AUC<sub>inf</sub>. The apparent elimination half-time for valnemulin in the lung was 3.57 h. The Limits of Quantitation (LOQ) was

Table 2: Lung and plasma concentrations and pharmacokinetic parameters following oral administration of 10 mg kg<sup>-1</sup> b.w. in pigs (study B)

Parameters	Lung	Plasma	Lung/plasma
AUC <sub>last</sub> (h*µg) g <sup>-1a</sup>	409.44	5.18	79.04
AUC <sub>inf</sub> (h*µg) g <sup>-1a</sup>	410.16	5.30	77.39
C <sub>max</sub> µg g <sup>-1</sup>	49.86	0.99	-
t <sub>max</sub> h	4.00	4.00	-
k <sub>e</sub> h <sup>-1</sup>	0.19	0.38	-
t <sub>1/2ke</sub> h	3.57	1.84	-

a = Units for plasma were (h\*µg) mL<sup>-1</sup>. AUC<sub>inf</sub> = AUC from time zero extrapolated to infinity. AUC<sub>last</sub> = Area under the moment curve computed to the last observation. C<sub>max</sub> = The maximum concentration. t<sub>max</sub> = The time to maximum concentration. k<sub>e</sub> = The elimination rate. t<sub>1/2ke</sub> = The elimination half-lives

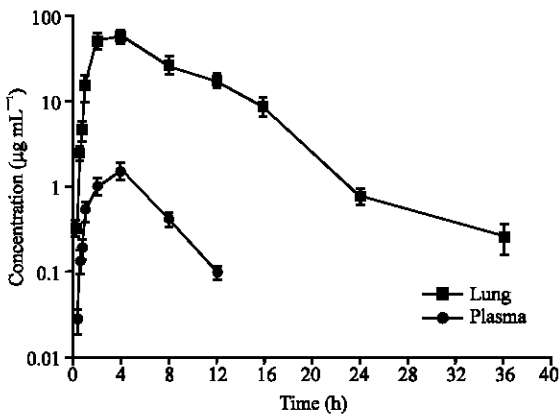


Fig. 2: Average concentration of valnemulin in pig lung tissue and plasma after oral administration of 10 mg kg<sup>-1</sup> b.w. (error bars indicate standard error) (study B)

10 ng mL<sup>-1</sup> (or 10 ng g<sup>-1</sup>) for both plasma and lung tissue samples. It meant that very low valnemulin concentrations could be measured. Therefore, a very selective and sensitive LC-MS/MS technique was used allowing quantification of valnemulin at low concentrations which was crucial to obtain reliable pharmacokinetic parameters.

After oral administration, variability at inflection time points was enhanced due to the variable presence of a secondary peak in the plasma concentration profile at approximately 6 h. The peak most likely correlates to enterohepatic recycling of valnemulin (Buur, 2007). The secondary peak was seen in four out of the ten pigs. The similar phenomenon was reported in broiler chickens following oral administration (Wang *et al.*, 2010). Enterohepatic recycling has been reported for valnemulin in pigs (Horkovics-Kovats and Schatz, 1997).

The investigation of elimination of [<sup>3</sup>H]-valnemulin in pigs confirmed that most of the drug is eliminated via the liver-bile-faeces route. The non-metabolized drug eliminated from the gall bladder is recycled (Horkovics-Kovats and Schatz, 1996). No side effects were observed after i.v. and p.o. administration at a

dosage of 10 mg kg<sup>-1</sup> b.w. in pigs. After valnemulin was administered intravenously in pigs, the disposition kinetic curve declined in a biphasic manner, revealing that plasma drug concentration-time profile was best described by a bi-exponential equation. However, the pharmacokinetic studies with a single i.v. valnemulin administration in chickens described non-compartmental model (Wang *et al.*, 2010). Intravenous administration resulted in a rapid elimination of valnemulin from the body (k<sub>e</sub> = 0.95±0.17 h<sup>-1</sup>).

The steady state volume of distribution (Vd<sub>ss</sub>) obtained in the current study was 5.28±0.35 L kg<sup>-1</sup>, suggesting that valnemulin might easily reach well perfused tissues (Bowman and Rand, 1980). Following p.o. administration (10 mg kg<sup>-1</sup> b.w.) to pigs, plasma drug concentrations were best fitted to a one-compartment model with a 1st order absorption rate. The absorption rate constant (k<sub>a</sub> = 0.34±0.03 h<sup>-1</sup>) was less than the elimination rate constant (k<sub>e</sub> = 1.05±0.19 h<sup>-1</sup>) which meant that the pharmacokinetic properties were absorption limited model (flip-flop). Therefore, it was important to administer a drug by the i.v. route to distinguish between k<sub>a</sub> and k<sub>e</sub> when the terminal half-life does not represent the drug elimination but rather drug absorption (Toutain and Bousquet-Melou, 2004). The valnemulin levels reached a C<sub>max</sub> of 0.59 µg mL<sup>-1</sup> in about 1.98 h. The similar result (C<sub>max</sub> = 0.66±0.15 µg mL<sup>-1</sup>) has been reported for valnemulin in broiler chickens (Wang *et al.*, 2010).

The high volume of distribution (10.79±1.49 L kg<sup>-1</sup>) indicated that valnemulin may be extensively distributed in pig tissues. A previous experiment in the laboratory to determine tissue distribution of valnemulin indicated that the lung and liver are the prime target tissues in swines. The previous study also proved the result (Horkovics-Kovats and Schatz, 1997; Huang *et al.*, 2010). After p.o. administration (10 mg kg<sup>-1</sup> b.w.) to pigs, valnemulin was extensively distributed to lung tissue. Peak concentrations in the lung were markedly higher (C<sub>max</sub> = 49.86 µg g<sup>-1</sup>) than in plasma (C<sub>max</sub> = 0.99 µg mL<sup>-1</sup>) and were reached 4 h (t<sub>max</sub>) postadministration. The lung AUC<sub>inf</sub> (410.16 h\*µg g<sup>-1</sup>) was about 77 times higher than the corresponding plasma AUC<sub>inf</sub> (5.3 h\*µg mL<sup>-1</sup>), suggesting that valnemulin may be extensively distributed in lung tissues. The lung t<sub>1/2ke</sub> (3.57 h) was longer than the corresponding plasma t<sub>1/2ke</sub> (1.84 h).

Bioavailability testing is a means of predicting the clinical efficacy of a drug. The systemic bioavailability (F = 57.43±3.89%) reported in the current study indicates good absorption of the drug following p.o. administration. Compared to previous studies, it is lower than the values determined in broiler chickens (74.42%) (Wang *et al.*, 2010) and Sprague-Dawley rats (100%).

## CONCLUSION

Based on results presented from these studies, valnemulin administered to pigs by the oral route demonstrated advisable bioavailability, extensive distribution to lung tissue, extended lung half-life. These pharmacokinetic profiles may be beneficial for an antimicrobial drug used for the treatment of mycoplasmal respiratory disease in swine.

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## REFERENCES

- Birch, A.J., C.W. Holzappel and R.W. Rickards, 1996. The structure and some aspects of the biosynthesis of pleuromutilin. *Tetrahedron*, 22: 359-387.
- Bowman, W.C. and M.J. Rand, 1980. Textbook of Pharmacology. 2nd Edn., Blackwell Scientific Publications, Oxford, pp: 39-25.
- Buur, J.L., 2007. Applications of physiologically based pharmacokinetic models in veterinary medicine. Ph.D. Thesis, North Carolina State University.
- Gabrielsson, J. and D. Weiner, 1997. Non-Compartmental Analysis in Pharmacokinetic / Pharmacodynamic Data Analysis: Concepts and Applications. 2nd Edn., Johan Gabrielsson and Daniel Weiner, Swedish Pharmaceutical Press, Stockholm, pp: 138-152.
- Gentry, D.R., L. McCloskey, M.N. Gwynn, S.F. Rittenhouse, N. Scangarella, R. Shawar and D.J. Holmes, 2008. Genetic characterization of Vga ABC proteins conferring reduced susceptibility to pleuromutilins in *Staphylococcus aureus* antimicrobial agents and chemotherapy. *Antimicrob. Agents Chemother.*, 52: 4507-4509.
- Hannan, P.C.T. and P.H. Ripley, 1996. *In vitro* development of resistance in *Mycoplasma hyopneumoniae* to SDZ PMD 296, tiamulin and two other antimicrobial agents. *Proc. Int. Congr. Pig. Vet. Soc.*, 14: 225-225.
- Hauschke, D., V. Steinijans and I. Pigeot, 2007. Basic Statistical Considerations. In: Bioequivalence Studies in Drug Development Methods and Applications, Hauschke, D., V. Steinijans and I. Pigeot (Eds.). John Wiley and Sons Ltd., San Francisco, USA., pp: 37-68.
- Hidalgo, A., A. Carvajala, C. Garcia-Feliza, J. Osorioa and P. Rubio, 2009. Antimicrobial susceptibility testing of Spanish field isolates of *Brachyspira hyodysenteriae*. *Res. Vet. Sci.*, 87: 7-12.
- Horkovics-Kovats, S. and F. Schatz, 1996. Physiologically based pharmacokinetic modelling with valnemulin and its metabolites after multiple oral administration in pigs. *J. Pharm. Med.*, 6: 149-167.
- Horkovics-Kovats, S. and F. Schatz, 1997. Pharmacokinetic modelling with valnemulin and its total metabolites after multiple oral administration to pigs. *J. Vet. Pharmacol. Therapeutics*, 20: 29-29.
- Hu, C.H. and Y. Zou, 2009. Mutilins derivatives: From veterinary to Human-used antibiotics. *Mini Rev. Med. Chem.*, 9: 1397-1406.
- Huang, Q., J. Li, L. Xia, X. Xia, P. Duan, J. Shen and S. Ding, 2010. Residue depletion of valnemulin in swine tissues after oral administration. *Anal. Chim. Acta*, 664: 62-67.
- Lobova, D., J. Smola and A. Cizek, 2004. Decreased susceptibility to tiamulin and valnemulin among Czech isolates of *Brachyspira hyodysenteriae*. *J. Med. Microbiol.*, 53: 287-291.
- Martinez, M.N., 1998. Noncompartmental methods of drug characterization: Statistical moment theory. *J. Am. Vet. Med. Association*, 213: 974-980.
- Riviere, J.E., 1999. Study Design and Data Analysis. In: Comparative Pharmacokinetics: Principles, Techniques and Applications, Riviere, J.E. *et al.* (Eds.). 1st Edn., Iowa State University Press, USA.
- Ross, R.F., 1999. Mycoplasmal Pneumonia of Swine. In: Disease of Swine, Straw, B.E., S. D'Allaire, W.L. Mengeling and D.J. Taylor (Eds.). 8th Edn., Iowa State University Press, Ames, Iowa, USA.
- Toutain, P.L. and A. Bousquet-Melou, 2004. Bioavailability and its assessment. *J. Vet. Pharmacol. Therapeutics*, 27: 455-466.
- Wang, R., L.G. Yuan, L.M. He, L.X. Zhu and X.Y. Luo *et al.*, 2010. Pharmacokinetics and bioavailability of valnemulin in broiler chickens. *J. Vet. Pharmacol. Therapeut.*, 34: 247-251.
- Yamaoka, K., T. Nakagawa and T. Uno, 1978. Application of Akaike's Information Criterion (AIC) in the evaluation of linear pharmacokinetic equations. *J. Pharmacokinetics Biopharmaceut.*, 6: 165-175.