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Residue Depletion of Imidocarb in Swine Tissue

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A residue depletion study was performed with high-performance liquid chromatography (HPLC) to determine a withdrawal period of imidocarb (IMD) in swine tissues. The drug was administered intramuscularly (im) at the dose of 2.0 mg kg^{-1} of body weight (bw) once a day for 3 days. Samples of muscle, fat, liver, kidney, and injection site muscle from 5 pigs were collected on 7, 14, 28, and 56 days after the last administration. Quantitative analysis of IMD was conducted by HPLC-UV at 260 nm after liquid−liquid extraction. The limit of detection (LOD) of the method was 0.1 μg g^{-1} for liver and kidney and 0.05 μg g^{-1} for muscle and fat, respectively. Mean recoveries of IMD in all fortified samples at a concentration range of 0.1−25 μg g^{-1} were 69.5−89.3%, with a coefficient of variation (CV) below 13.3%. In swine, the highest drug levels occurred in liver and kidney during the whole study period, suggesting that these tissues are targets for residues. IMD concentrations in all examined tissues were below the accepted maximum residue limits (MRLs) recommended by the Committee for Veterinary Medical Products (CVMP) of the European Medical Evaluation Agency (EMEA) at 54 days post-treatment.

KEYWORDS: Imidocarb; swine; residue depletion; intramuscular administration; HPLC-UV

INTRODUCTION

Imidocarb (IMD) is a chemotherapeutic and chemoprophylactic agent belonging to the family of carbanilide derivatives [3,3′-bis(2-imidazolin-2-yl)-carbanilide] with antiprotozoal activity. The structure is shown in **Figure 1**. IMD is usually administered as dipropionate salt (IMDP) and has been successfully used as a therapeutic or prophylactic agent against babesiosis in cattle (*1*−*3*), lambs (*4*), sheep (*5*), horses (*6*), and dogs (*7*, *8*) and also commonly used against anaplasmosis in bovine (*9*, *10*), against ehrlichiosis in dogs (*11*, *12*), against leishmaniasis in mice (*13*), against theileriosis in cattle (*14*, *15*), and against eperythrozoonosis in sheep (*16*).

Eperythrozoonosis is an important intercurrent disease and an emerging swine disease that has spread throughout all provinces in China in recent years, which has been proven to relate to the reproductive failure in sows, icteroanemia in pigs of various ages, and poor performance in chronically affected swine. Recently, it was reported that IMDP had showed a favorable kinetic profile in swine, such as rapid absorption, wide distribution, long half-life of elimination, and acceptable bioavailability (*17*) and had a chemotherapeutic efficacy against eperythrozoonosis in swine at the dosage of 2.0 mg kg^{-1} body weight (bw) intramuscularly (im) or intravenously (iv) (*18*). However, little is known about its residue depletion in swine following im administration. Therefore, the objective of this study was to obtain the residue data of IMDP in swine tissues and establish a reasonable drug withdrawal period that is safe for human consumption.

MATERIALS AND METHODS

Reagents and Materials. IMDP injection (5 g/100 mL) oil formulation was kindly provided by Qilu Animal Health Products Corp. Ltd. (Jinan, Shandong, China). Acetonitrile (ACN) and methanol from Fisher Scientific (Fair Lawn, NJ) were high-performance liquid chromatography (HPLC)-grade. Trifluoroacetic acid was obtained from J.T. Baker (Phillipsburg NJ), and Tris(hydroxymethyl)aminomethane was purchased from Promega Corp. (Madison, WI). Subtilisin Carlsberg was

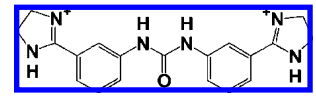

Figure 1. Molecular structure of IMD.

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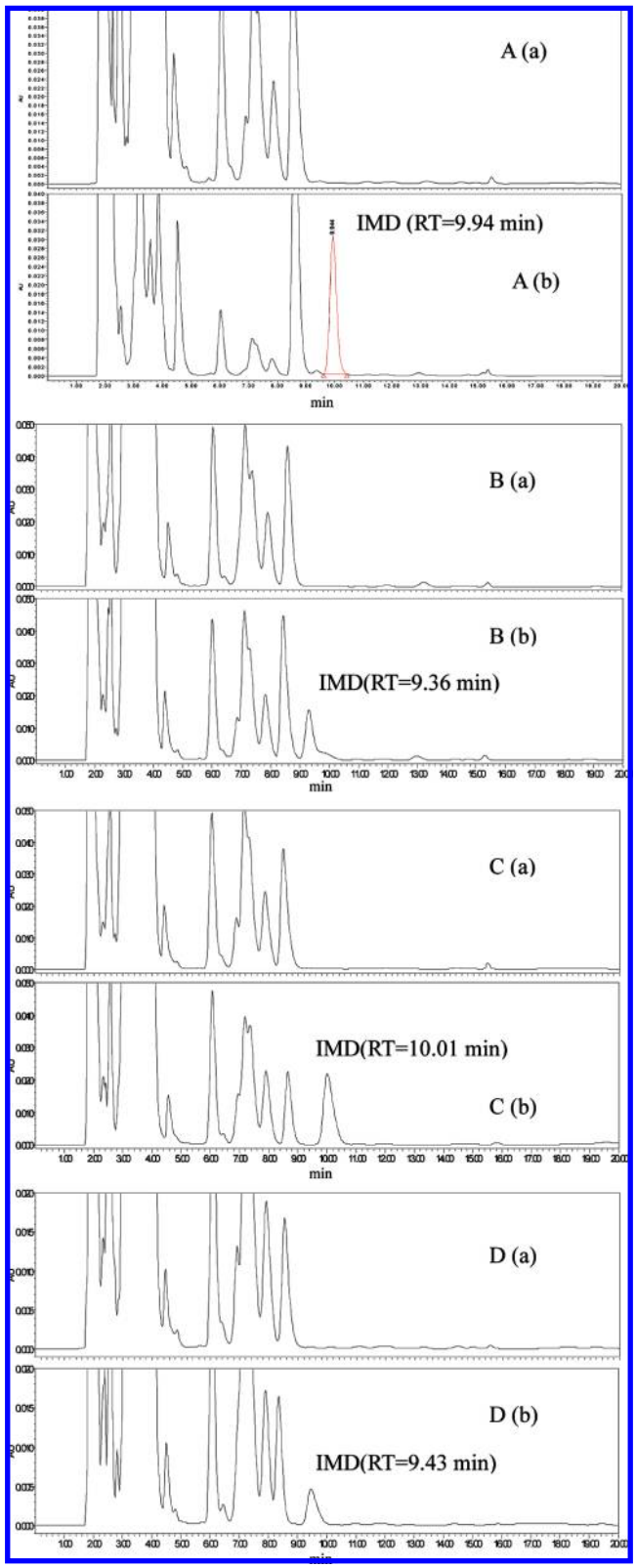

Figure 2. Chromatograms of (A) liver, (B) kidney, (C) muscle, and (D) fat: (a) blank of the sample and (b) spiked sample. A (a) Fortification at 2.5 μg g^{-1}, B (a) fortification at 1.0 μg g^{-1}, C (a) fortification at 1.0 μg g^{-1}, and D (a) fortification at 0.5 μg g^{-1}.

from Sigma (Poole, Dorset, U.K.). All other chemicals used as reagents in the assay (analytical grade) were obtained from Beijing Chemical Reagent Corp. (Beijing, China). Water was obtained from a Simpak 2 system (Millipore Corporation, Bedford, MA) during the whole study. Before HPLC analysis, all solutions were filtered through a 0.45 μm hydrophilic polypropylene membrane filter (Pall Corp., East Hills, NY).

Table 1. Mean Recoveries and Precisions of IMD in Fortified Tissue Samples ($n = 5$)

sample	fortification (μg g^{-1})	recovery (%) (mean ± SD)	intraday CV[a] (%)	interday CV (%)
liver	0.2	87.62 ± 1.35	1.54	5.23
	2.5	83.90 ± 1.77	2.11	4.99
	25.0	80.40 ± 3.41	4.24	7.36
kidney	0.2	86.02 ± 3.35	3.90	5.69
	1.0	84.91 ± 2.20	2.59	4.71
	5.0	81.49 ± 3.71	4.55	8.24
muscle	0.1	85.41 ± 4.70	5.50	7.66
	1.0	82.81 ± 5.92	7.22	4.83
	3.0	81.43 ± 2.38	2.91	6.05
fat	0.1	80.26 ± 3.32	4.14	8.51
	0.5	74.56 ± 4.18	5.61	7.77
	1.0	69.49 ± 3.61	5.20	13.44
injection muscle	0.1	89.33 ± 1.81	2.03	7.66
	1.5	85.65 ± 3.78	4.41	5.87
	5.0	80.11 ± 3.27	4.08	6.98

[a] CV = coefficient of variation.

Tris buffer (1 M) was prepared by adding 121.14 g of Tris into 800 mL of water, and then the volume was made up to 1000 mL when dissolved. It was passed through a 0.20 μm membrane and degasified before use. Buffer was stored at 4 °C and used within a week after preparation.

Standard. The IMD reference standard (98% purity) was supplied by the China Institute of Veterinary Drug Control (Beijing, China). A stock solution of 1000 μg mL^{-1} was prepared by dissolving 10 mg of IMD standard in 10 mL of water. The solution was stored at 4 °C and was stable for at least 1 month. Working standard solutions of IMD were prepared by diluting the standard solution with the mobile phase.

Sample Analysis. A previously described extraction method was used to measure IMD in the different matrices analyzed (*19*), with some minor modifications. Briefly, the sample (8 g) was homogenized in 16 mL of 1 M Tris (pH 10.5, $\rho = 1.03$ g mL^{-1}). Homogenized sample (15.3 g, corresponding to 5 g of original tissue) was weighted with a fresh tube, digested by incubation with 10 mg of subtilisin, vortex-mixed for 1 min, and placed in a thermostatic bath for 1 h at 56 °C, mixing every 10 min to ensure complete degradation of the tissue. After enzymatic digestion, the sample was adjusted to pH 1 with 2 mL of 6 M HCl, vortex-mixed for 2 min, and centrifuged (8000*g* for 15 min). Supernatant was decanted with a fresh tube, added to 5 g of NaCl, basified with 4 mL of 10 M NaOH, extracted twice with 20 mL of hexane/isoamyl alcohol (3:2, v/v) by shake mixed for 10 min, ultrasonicated for 20 min at 40 °C, and centrifuged (8000*g* for 15 min). The organic phases were pooled and re-extracted with 2 mL of 1 M HCl by vortex mixing for 1 min, ultrasonicated for 20 min at 40 °C, and centrifuged (8000*g* for 15 min). The acidified aqueous phase was collected and filtered through a 0.45 μm nylon syringe filter. Extracts (20 μL) was analyzed by HPLC (Waters 2695, Milford, MA) using a mobile phase of 0.1% trifluoroacetic acid in water/acetonitrile at a fan 88:12 (v/v) ratio (*17*) at a flow rate of 1 mL min^{-1} on a 150 × 4.6 mm column (5 μm, XBridge C18 RP column, Waters, Milford, MA), equipped with an appropriate guard column. The column temperature was maintained at 30 °C, and the UV detector (Waters 2487, Milford, MA) was set at 260 nm (*20*).

Analyte Recovery Studies. To test the stability of the HPLC system and the feasibility of the detection method for IMD in swine tissues, a fortifying test was conducted on muscle, fat, liver, and kidney at different fortification levels. The precision (inter- and intraday) of the method was assessed using five replicates of control and fortified samples at three fortification levels on 3 different days.

Method Calibration. The calibration curves were prepared with the peak areas and the working standard solution concentration. The standard curve for IMD was constructed with standard working solution concentrations of 0.01, 0.05, 0.1, 0.5, 1.0, 5.0, 10.0, and 50.0 μg mL^{-1}.

Animals Treatment. A total of 25 clinically healthy cross-bred (Duroc × Landrace × Yorkshire) pigs weighing 19−21 kg were

Table 2. Residue Concentrations of IMD in Swine Tissues after Three Intramuscular Injections at a Dose of 2.0 mg kg^{-1} bw with 24 h Intervals ($n = 5$) ($\mu g\ g^{-1}$)

	liver	kidney	muscle	fat	injection muscle
7 days	9.245 ± 3.634	2.253 ± 1.193	0.778 ± 0.604	0.38 ± 0.21	0.864 ± 0.413
14 days	5.378 ± 1.520	0.884 ± 0.039	0.209 ± 0.089	0.084 ± 0.032[a]	0.285 ± 0.134
28 days	2.719 ± 1.201	0.577 ± 0.456	ND[b]	ND	0.121 ± 0.083
56 days	0.823 ± 0.174	ND	ND	ND	ND

[a] Average value of three pigs. [b] ND = not detected.

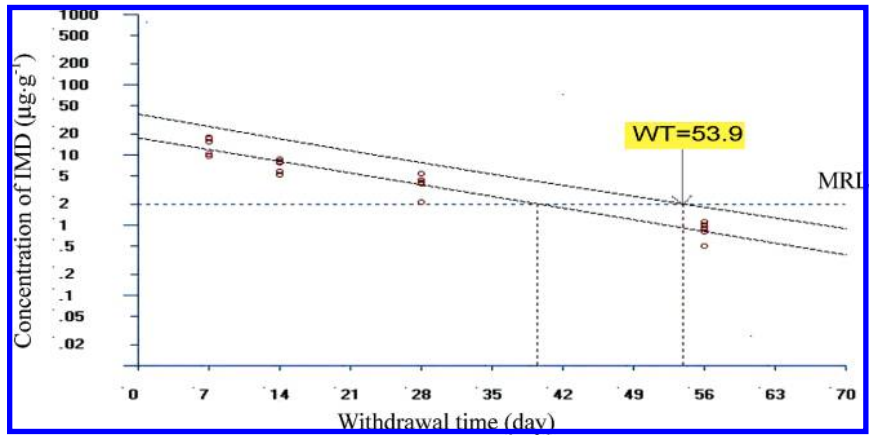

Figure 3. Plot of withdrawal time calculation for swine liver at the time when the one-sided 95% upper tolerance limit was below the MRL of 2.0 $\mu g\ g^{-1}$.

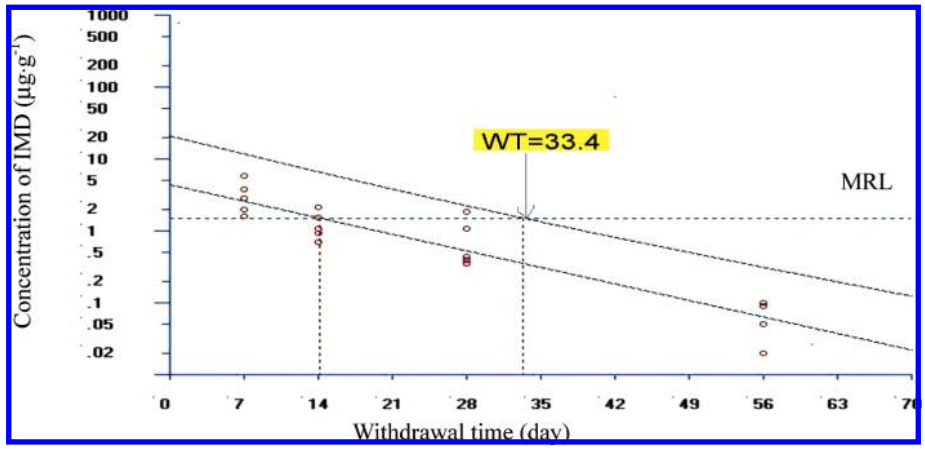

Figure 4. Plot of withdrawal time calculation for swine kidney at the time when the one-sided 95% upper tolerance limit was below the MRL of 1.5 $\mu g\ g^{-1}$.

used for treatment. The animals had not received any treatment in the last month prior to the study. They were bred in experimental cages, had access to tap water from drinking nipples, and were fed *ad libitum* on a conventional feed without antibiotics during the whole study. All pigs were ear-tagged, and the site of injection at the back of the ear was sheaved and marked with indelible ink. They were acclimated to experimental conditions for at least 1 week prior to initiation of the study. Five animals were kept as the control. A total of 20 animals were weighed on the same day and injected 3 times on the right marked injection site of the neck at a dose of 2.0 mg kg^{-1} bw within 24 h intervals. Groups of five pigs were slaughtered by captive-bolt stunning and exsanguinations at 7, 14, 28, and 56 days after the last dose. The tissues were collected from each pig: a 300−400 g (approximate) core at a radius of 7.5 cm from injection sites 2 and 3 for residue analysis; the entire liver; both kidneys; 250−500 g of muscle from the left semimembranosus/semitendinosus muscles, and 100−300 g of subcutaneous fat. Tissues were transferred to a −20 °C freezer immediately after collection. Then, they were thawed for processing to produce a homogeneous ground sample and stored at −80 °C until analysis.

RESULTS AND DISCUSSION

Method Validation. Under the adopted analytical conditions, IMD eluted with a retention time of about 10 min and the other peaks on the matrix were completely separated from IMD. The calibration curve of the detector response was linear over the selected concentration range (0.01−50 $\mu g\ mL^{-1}$). The correlation coefficients (R) of standard curves were 0.9999. The limit of detection (LOD) and the limit of quantification (LOQ) of the HPLC method were defined on the basis of signal-to-noise (S/N) ratios of 3:1 and 6:1, respectively (*21*). In this depletion study, the LOD and LOQ were 0.1 and 0.2 $\mu g\ g^{-1}$ for liver and kidney and 0.05 and 0.1 $\mu g\ g^{-1}$ for muscle and fat, respectively. The typical chromatograms of IMD standard, blank, and fortified samples are shown in **Figure 2**. IMD was well-detected by this HPLC method with UV detection at 260 nm, which indicated high selectivity of the method. To evaluate the accuracy and reproducibility of the method, fortified samples at three different concentrations on 3 different days for each tissue were assessed

using blank swine tissues spiked with IMD. Concentrations added to tissues, percentages of recovery, and inter- and intraday coefficients of variation of the analytical method are reported in **Table 1**. The validation parameters obtained for the analytical procedure selection for detecting IMD in swine tissues were in good agreement with the validation criteria recommended by the European Union and ensured that these procedures were suitable for the intended purpose.

Residue Depletion Study. In this residue study, the incurred swine tissues were collected from 20 pigs, which were divided into five groups randomly. Mean concentrations with SD of IMD in liver, kidney, muscle, fat, and injection muscle from swine administered im 3 times at a dose of 2.0 mg per kg of bw in 3 consecutive days and slaughtered at 7, 14, 28, and 56 days post-treatment were summarized in **Table 2**. In muscle and fat samples, IMD was not detected at 28 and 56 days post-treatment. Higher residue concentrations of IMD were detected in muscle samples from injection sites than that in semimembranosus/semitendinosus muscle samples, and the average the residue concentrations was 0.121 ± 0.083 $\mu g\ g^{-1}$ at 28 days post-administration. At 7 days post-administration, the average IMD concentrations of 9.245 ± 3.634 $\mu g\ g^{-1}$ in liver and 2.253 ± 1.193 $\mu g\ g^{-1}$ in kidney were measured. At 56 days post-treatment, the average concentrations of IMD in liver tissue declined to 0.823 ± 0.174 $\mu g\ g^{-1}$ and all of the concentrations of IMD in all collected kidney samples were not detected. The depletion curve (**Figures 3** and **4**) was prepared with the average residue concentrations in swine liver and kidney samples and the withdrawal time. The comparison of two curves clearly showed that the rates of IMD residue elimination from liver and kidney were similar from 7 to 28 days post-administration.

These results suggest that the highest levels of the drug in swine occur in the liver and kidney, indicating that these are target tissues for IMD residue, which is consistent with the data reported for sheep, horses, and cows (*22*−*25*). Although the concentrations of IMD in swine muscle were always lower than the liver and kidney concentrations, muscle proved to have some importance as a storage tissue for IMD.

In 1998, the European Medical Evaluation Agency (EMEA) published the conclusions and recommendations of the Committee for Veterinary Medical Products (CVMP) with the provisional maximum residue limits (MRLs) (expiry 1.1.2002) for IMD in edible tissue of cattle and sheep as 0.300 $\mu g\ g^{-1}$ for muscle, 0.050 $\mu g\ g^{-1}$ for fat, 2.0 $\mu g\ g^{-1}$ for liver, 1.5 $\mu g\ g^{-1}$ for kidney, and 0.05 $\mu g\ g^{-1}$ for milk (*26*−*28*). Even if no MRLs had been established for the edible tissues of swine, the existing MRLs for cattle and sheep may be extrapolated to swine, according to the guidance on the establishment of MRLs for many animal species. As shown in **Table 2** and **Figures 3** and **4**, the concentrations of IMD residues in kidney, muscle, fat, and injection muscle from swine im administered 3 times at a dose of 2.0 mg kg^{-1} of bw with 24 h intervals were below the MRLs at 14 days of withdrawal time, except for the residue in liver at 40.3 days. Because of the limited number of test animals, high animal individual variability, and potential hazards to human health, the withdrawal periods were established on the basis of MRLs using the statistical method (95% tolerance limit and 95% confidence) stated in the guidance (*29*), which were 53.9 days for liver and 33.4 days for kidney. Because the time points do not make up a full day, the withdrawal periods have to be rounded up to the next day. Therefore, the longest withdrawal time of 54 days for liver and 34 days for kidney can be selected as the conclusive withdrawal time to guarantee consumer safety. This prolonged drug persistence in the animal body may be ascribable to resistance to biotransformation (*17*, *22*, *31*) and strong binding to nuclear components, thus causing the formation of large deposits, especially to DNA in tissues with higher DNA content (primarily in liver and kidney) (*17*, *19*, *23*). Moreover, the affinity and capacity of the binding with DNA is much higher when compared to other macromolecules, including α_1-acid glycoprotein, bovine serum albumin (BSA), or hemoglobin (*19*, *22*). This high drug concentration maintained in target tissues may supply the animal body with delivery reservoirs for the continuous release of IMD and produce low plasma concentrations at later stages of treatment (*24*, *25*).

In conclusion, an HPLC-UV method was introduced for the determination of IMD in swine liver, kidney, muscle, fat, and injection muscle. The results indicate the tissue distribution and residue depletion characteristics of IMD in swine tissues after multiple im dosing, which can provide a scientific basis for administering IMD in clinical practice and recommending a rational withdrawal period and safety assurance for human food consumption. The residue levels in swine tissue were already below the MRLs established at the second sampling for muscle, fat, and injection muscle and depleted to less than the MRLs in kidney within 34 days after dosing, while 54 days were needed to attain tolerable concentrations in the liver.

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LITERATURE CITED

(1) Kuttler, K. L.; Johnson, L. W. Chemoprophylactic activity of imidocarb, diminazene and oxytetracycline against *Babesia bovis* and *B. bigemina*. *Vet. Parasitol*. **1986**, *21* (2), 107–118.

(2) Kuttler, K. L. The effect of imidocarb treatment on *Babesia* in the bovine and the tick (*Boophilus microplus*). *Res. Vet. Sci*. **1975**, *18*, 198.

(3) Thompson, K. C.; Todorovic, R. A.; Mateus, G. Methods to improve the health of cattle in the tropics: Immunisation and chemoprophylaxis against haemoparasitic infections. *Trop. Anim. Health Prod*. **1978**, *10* (2), 75–81.

(4) Sevinc, F.; Turgut, K.; Sevinc, M.; Ekici, O. D.; Coskun, A.; Koc, Y.; Erol, M.; Ica, A. Therapeutic and prophylactic efficacy of imidocarb dipropionate on experimental *Babesia ovis* infection of lambs. *Vet. Parasitol*. **2007**, *149* (1−2), 65–71.

(5) McHardy, N.; Woollon, R. M.; Clampitt, R. B.; James, J. A.; Crawley, R. J. Efficacy, toxicity and metabolism of imidocarb dipropionate in the treatment of *Babesia ovis* infection in sheep. *Res. Vet. Sci*. **1986**, *41*, 14–20.

(6) Frerichs, W. M.; Allen, P. C.; Holbrook, A. A. Equine piroplasmosis (*Babesia equi*): Therapeutic trials of imidocarb dihydrocloride in horses and donkeys. *Vet. Rec*. **1973**, *93*, 73–75.

(7) Bourdoiseau, G. Canine babesiosis in France. *Vet. Parasitol*. **2006**, *138* (1−2), 118–125.

(8) Penzhorn, B.; Lewis, L.; de Waal, B. D.; López Rebollar, D. T. Sterilization of *Babesia canis* infections by imidocarb alone or in combination with diminazene. *J. S. Afr. Vet. Assoc*. **1995**, *66* (3), 157–159.

(9) Wilson, A. J.; Parker, R.; Parker, M.; Hall, W. T.; Trueman, K. F. Chemotherapy of acute bovine anaplasmosis. *Aust. Vet. J*. **1979**, *55* (2), 71–73.

(10) Coetzee, J. F.; Apley, M. D.; Kocan, K. M.; Jones, D. E. Flow cytometric evaluation of selected antimicrobial efficacy for clearance of *Anaplasma marginale* in short-term erythrocyte cultures. *J. Vet. Pharmacol. Ther*. **2006**, *29* (3), 173–183.

(11) Matthewman, L. A.; Kelly, P. J.; Brouqui, P.; Raoult, D. Further evidence for the efficacy of imidocarb dipropionate in the treatment of *Ehrlichia canis* infection. *J. S. Afr. Vet. Assoc*. **1994**, *65* (3), 104–107.

(12) Price, J. E.; Dolan, T. T. A comparison of the efficacy of imidocarb dipropionate and tetracycline hydrochloride in the treatment of canine ehrlichiosis. *Vet. Rec*. **1980**, *107* (12), 275–277.

(13) Rodrigues, F. H.; Afonso-Cardoso, S. R.; Gomes, M. A.; Beletti, M. E.; Rocha, A.; Guimarães, A. H.; Candeloro, I.; de Souza, M. A. Effect of imidocarb and levamisole on the experimental infection of BALB/c mice by *Leishmania* (*Leishmania*) *amazonensis*. *Vet. Parasitol*. **2006**, *139* (1−3), 37–46.

(14) Purnell, R. E.; Rae, M. C.; Deuk, S. M. Efficacy of imidocarb diproprionate and primaquine phosphate in the prevention of tick-borne disease in imported Hereford heifers in South Korea. *Trop. Anim. Health Prod*. **1981**, *13* (3), 123–127.

(15) Purnell, R. E.; Rae, M. C. The use of imidocarb diproprionate for the treatment of *Theileria sergenti* infections of cattle. *Aust. Vet. J*. **1981**, *57* (5), 224–226.

(16) Hung, Ch. A. L. Chemotherapeutic efficacy of imidocarb dipropionate on experimental *Eperythrozoon ovis* infection in sheep. *Trop. Anim. Health Prod*. **1986**, *18*, 97–102.

(17) Su, D.; Li, X. B.; Wang, Z. J.; Wang, L.; Wu, W. X.; Xu, J. Q. Pharmacokinetics and bioavailability of imidocarb dipropionate in swine. *J. Vet. Pharmacol. Ther*. **2007**, *30* (4), 366–370.

(18) Song, J. C.; Zhang, S. F.; Xu, Y. T.; Jin, C. M. Comparative efficacy of diminazene diaceturate, imidocarb dipropionate and erythromycin thiocyanate against *Eperythrozoon suis* infections in swine. *Heilongjiang, J. Anim. Sci. Vet. Med*. **2003**, *8*, 50–51.

(19) Coldham, N. G.; Moore, A. S.; Sivapathasundaram, S.; Sauer, M. J. Imidocarb depletion from cattle liver and mechanism of retention in isolated bovine hepatocytes. *Analyst* **1994**, *9*, 2549–2552.

(20) Coldham, N. G.; Moore, A. S.; Dave, M.; Graham, P. J.; Sivapathasundaram, S.; Lake, B. G.; Sauer, M. J. Imidocarb residues in edible bovine tissues and in vitro assessment of imidocarb metabolism and cytotoxicity. *Drug Metab. Dispos*. **1995**, *23*, 501–505.

(21) Tarbin, J. A.; Tyler, D.; Shearer, G. The use of ion-exchange as a clean-up procedure for the trace residue analysis of veterinary drugs in animal tissues. Residues Veterinary Drugs Food, Proceedings of the 2nd European Residue Conference, 1993; Vol. 2, pp 659−663.

(22) Aliu, Y. O.; Davis, R. H.; Camp, B. J.; Kuttler, K. L. Absorption, distribution and excretion of imidocarb dipropionate in sheep. *Am. J. Vet. Res*. **1977**, *38*, 2001–2007.

(23) Abdullah, A. S.; Baggot, J. D. Pharmacokinetics of imidocarb in normal dogs and goats. *J. Vet. Pharmacol. Ther*. **1983**, *6*, 195–199.

(24) Lai, O.; Belloli, C.; Crescenzo, G.; Ormas, P. Depletion and bioavailability of imidocarb residues in sheep and goat tissues. *Vet. Hum. Toxicol*. **2002**, *44* (2), 79–83.

(25) Belloli, C.; Crescenzo, G.; Lai, O.; Carofiglio, V.; Marang, O.; Ormas, P. Pharmacokinetics of imidocarb dipropionate in horses after intramuscular administration. *Equine Vet. J*. **2002**, *34* (6), 625–629.

(26) U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), Center for Veterinary Medicine (CVM). Guidance for Industry, Bioanalytical Method Validation. May, 2001 (available at http://www.fda.gov/cder/guidance/index.htm).

(27) CVMP Imidocarb Summary Report (1). EMEA/MRL/444/98-FINAL (www.emea.eu.int). Committee for Medicinal Products, European Medicines Agency, London, U.K., 1998.

(28) CVMP Guideline on Validation of Analytical Procedures: Methodology. EMEA/CVMP/VICH/591/98-FINAL (www.emea.eu.int). Committee for Medicinal Products for Veterinary Use, European Medicines Agency, London, U.K., 1998.

(29) CVMP Imidocarb (Extension to Sheep). Summary Report. EMEA/MRL/881/03-FINAL (www.emea.eu.int). Committee for Medicinal Products for Veterinary Use, European Medicines Agency, London, U.K., 2003.

(30) Committee for Veterinary Medicinal Products (CVMP). The European Agency for the Evaluation of Medicinal Products. Note for Guidance: Approach towards Harmonization of Withdrawal Period, EMEA/CVMP/036/95, 1995.

(31) Moore, A. S.; Coldham, N. G.; Sauer, M. J. A cellular mechanism for imidocarb retention in edible tissues. *Toxicol. Lett*. **1996**, *87*, 61–68.

(32) Kuttler, K. L.; Johnson, L. W. Chemoprophylactic activity of imidocarb, diminazene and oxytetracycline against *Babesia bovis* and *B. bigemina*. *Vet. Parasitol*. **1986**, *21* (2), 107–118.

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