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ARTICLE

Tissue Depletion and Concentration Correlations between Edible Tissues and Biological Fluids of 3-Amino-2-oxazolidinone in Pigs Fed with a Furazolidone-Medicated Feed

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Furazolidone has been prohibited for use in food animal production worldwide for its carcinogenicity and mutagenicity, but it is still illegally used in some farms because of its effectiveness and cheap price. Because of the food safety and economical concerns, it is necessary to find an efficient and low-cost way to monitor the misuse of furazolidone in food-producing animals. For this regard, the tissue depletion and tissue−biological fluid concentration correlations of 3-amino-2-oxazolidinone (AOZ), which is the marker residue of furazolidone, were studied in pigs. Pigs were dosed with 400 mg/kg of furazolidone in feed for 7 days and were sacrificed at the withdrawal time of 0.5, 7, 21, 35, 56, and 63 days. Muscle, liver, kidney, urine, and plasma were collected to detect the AOZ by a simplified indirect competitive enzyme-linked immunosorbent assay (ic-ELISA). Results showed that AOZ was widely distributed in pigs and eliminated slowly after the digestion of furazolidone. The half-lives of AOZ in the plasma, urine, liver, kidney, and muscle were 13.7, 14.7, 13.6, 13.6, and 15.0 days, respectively. Good correlations of the AOZ concentration were found between plasma and muscle, plasma and liver, urine and liver, and urine and kidney in the depletion period of 7−63 or 21−63 days, with correlation coefficients of more than 0.97 and p values less than 0.05. These correlations can provide a basis for a simple and economical way using plasma/urine to monitor the illegal use of furazolidone in pigs without slaughter.

KEYWORDS: Furazolidone; 3-amino-2-oxazolidinone; biological fluids; tissue depletion

INTRODUCTION

Furazolidone (**Figure 1**) belongs to the group of nitrofuran antibacterial agents. It was widely used as an antimicrobial for the treatment of gastrointestinal infections and also as a growth promoter for the improvement of production efficiency in animal husbandry. Because of its carcinogenicity and mutagenicity (*1*), furazolidone has been prohibited for use in food-producing animals worldwide (*2*, *3*). However, there are indications of its illegal use in many parts of the world for its cheapness and effectiveness (*4*), which has a negative impact on food safety and international trades and prompts many countries to initiate furazolidone-testing schemes.

Detecting the parent drug has no practical significance for the residue monitoring of furazolidone, because it is rapidly metabolized and excreted in the body. No parent drug could be detected at 12 h after administration in edible tissues (*5*). 3-Amino-2-oxazolidinone (AOZ; **Figure 1**) is recognized as the marker residue of furazolidone (*6*−*9*). Therefore, the effective control of furazolidone residues in edible tissues from treated animals is focused on the detection of AOZ.

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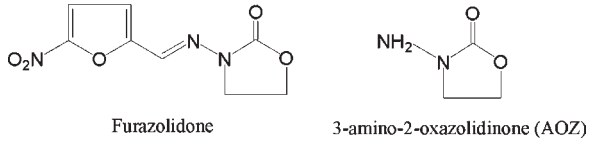

Figure 1. Chemical structures of furazolidone and 3-amino-2-oxazolidinone (AOZ).

In general, drug residue detection is based on testing of the edible tissues because they are digested directly by the consumer. To obtain edible tissues for residue monitoring, the animal must be sacrificed, which is an economical concern for the producers. Therefore, a pre-slaughter live monitoring strategy would prevent such waste. How to actualize the pre-slaughter drug residue monitoring is significant work to be done. Biological fluids, such as urine and plasma, are more practicable and convenient for pre-slaughter sampling. Determining correlations of drug residues between edible tissues and biological fluids is a prerequisite for developing the pre-slaughter residue monitoring. The animals can be marketed if the monitoring from the fluid predicates no residues existing or residue levels below maximum residue limits (MRLs) in

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the edible tissues. Alternatively, a longer withdrawal period must be followed if residue levels predicated in edible tissues are higher than the MRLs. This kind of predicating the misuse of an illegal drug would lead to an immediate discard of the animals treated.

The relationship of tissue−biological fluid drug concentrations in animals demonstrated the feasibility of using biological fluids to monitor drug residues in food-producing animals (*10*−*16*). Few studies on the AOZ residue depletion in edible tissues in pigs showed that AOZ persisted in swine tissues for 52 days after drug administration (*8*, *17*, *18*). One group studied the depletion of AOZ in pig urine and indicated a relationship between kidney and urine, suggesting a possible use of biological fluid to monitor the furazolidone residue (*8*). Further investigations are needed to study the exact correlation of the AOZ concentration between the edible tissues and biological fluids.

The aim of the present study was to obtain residue elimination data of AOZ in edible tissues, plasma, and urine of pigs, to establish correlations of AOZ residue between tissues and plasma/urine. Appropriate biological fluid (plasma or urine) might be chosen as a new matrix for monitoring the furazolidone residue in the field based on these correlations. Liquid chromatography−tandem mass spectrometry (LC−MS/MS) has been considered as a definitive or reference procedure for furazolidone residue detection (*18*−*20*), but the problem is that it cannot be conveniently introduced into a local farm or slaughterhouse to monitor the drug residues. Of the procedures cited, enzyme immunoassay is probably more suitable for routine monitoring of AOZ residues because of its rapid, reliable, and inexpensive nature (*21*−*24*). This work resulted in a new method for the pre-slaughter drug residue monitoring for furazolidone in swine, using readily available biological fluids, urine and plasma.

MATERIALS AND METHODS

Drugs and Chemicals. Furazolidone with a purity of >98.0% was purchased from Shangdong Jintai Co., Ltd. (Jinan, China). AOZ with a purity of >99.0% was purchased from WITEGA Laboratorien (Berlin, Germany). *N*-[Phenzlidene]-3-amino-2-oxazolidinone (PAOZ) with a purity of >99.0% and AOZ antibody against PAOZ were obtained from the National Reference Laboratory of Veterinary Drug Residues (HZAU) (Wuhan, China) (*22*). All other chemicals were of analytical grade.

Animals. The use of animals in the present study was performed in accordance with the Guide for the Care and Use of Laboratory Animals provided by the Institutional Animal Care. A total of 30 healthy large Landrace-Large white crossbred castrated male pigs weighing 15−18 kg were purchased from the Breeding Pigs Testing Center (Wuhan, China). The pigs were acclimated for 1 week prior to study. All pigs were offered a standard ration feed based on corn and soybean *ad libitum* during the duration of the study. Fresh water was available all of the time, and pens were cleaned daily.

The pigs were randomly divided into a control group ($n = 6$) and a test group ($n = 24$). The control group was fed with a standard ration based on corn and soybean containing no furazolidone. The test group was fed a medicated feed *ad libitum* for 7 consecutive days. The medicated feed contained a standard ration premixed with furazolidone at a level of 400 mg/kg, which was the recommended therapeutic dose for furazolidone preparations (*25*).

Sampling. On days 0.5, 7, 21, 35, 56, and 63 following withdrawal of the medicated feed, one pig in the control group and four pigs in the test group were euthanized by captive bolt according to the American Veterinary Medical Association (AVMA) guidance for euthanasia (*26*). Blood samples (10 mL) were collected from the right or left jugular vein using disposable heparinized vacutainer tubes. Plasma were prepared by placing the blood sample in an ice bath for 2 h and centrifuged at 25 °C for 10 min at 1100*g* ($r = 107$ mm). Edible tissue samples as longissimus dorsi muscle, liver, and kidney were collected and placed in plastic bags on an ice bath. Urine samples were collected daily after the last dosing. All samples were assayed immediately or were frozen at −20 °C until the analysis.

Sample Preparation. *Plasma*. A total of 0.5 mL of plasma was poured into 10 mL disposable plastic centrifuge tubes. To make a matrix-matched calibration curve, blank plasma was added with AOZ standard solution in 0.1 M phosphate-buffered saline (PBS) (pH 7.4) to obtain concentrations of 0, 1, 10, 40, 160, 640, and 2560 μg/L in plasma. A total of 2.5 mL of 0.1 M PBS (pH 7.4) was added into tubes, and 150 μL of 5 M HCl and 50 μL of 0.05 mol/L benzaldehyde (BA) in dimethyl sulfoxide (DMSO) were added and incubated at 37 °C for 12 h. A total of 200 μL of 20% trichloroacetic acid in water was added into each tube and vortexed thoroughly for 30 s. After centrifugation at 12000*g* ($r = 107$ mm) at 25 °C for 10 min, supernatants were transferred into tubes. The final pH was adjusted to 7.1−7.2 by the addition of 2 M NaOH on a pH-checker. The solutions were ready for enzyme-linked immunosorbent assay (ELISA) detection.

Urine. A total of 1 mL of urine was poured into 10 mL disposable plastic centrifuge tubes. To make a matrix-matched calibration curve, blank urine was added with AOZ standard solution in 0.1 M PBS (pH 7.4) to obtain concentrations of 0, 1, 10, 40, 160, 640, and 2560 μg/L in urine. A total of 3 mL of PBS, 150 μL of 5 M HCl, and 50 μL of 0.05 mol/L BA were added and incubated at 37 °C for 12 h. A total of 0.35 mL of 2 M NaOH was added into each tube and vortexed thoroughly for 30 s. The pH was adjusted to 7.1−7.2 by the addition of 1 M NaOH on a pH-checker. The supernatants were transferred into tubes and were ready for ELISA detection.

Edible Tissues. Muscles, kidneys, and livers were minced and homogenized. The homogenized samples (1.00 ± 0.01 g) were weighed and put into 10 mL disposable plastic centrifuge tubes. To make a matrix-matched calibration curve, blank tissues were added with AOZ standard solution in 0.1 M PBS (pH 7.4) to obtain concentrations of 0, 1, 10, 40, 160, 640, and 2560 μg/L in tissues. A total of 3 mL of water was added into tubes and incubated at 80 °C for 1 h in a water bath. Samples were cooled to room temperature, and 150 μL of 5 M HCl and 50 μL of 0.05 mol/L BA were added and incubated at 37 °C for 12 h. A total of 0.35 mL of 2 M NaOH was added into each tube and vortexed for 30 s. The pH was adjusted to 7.1−7.2 by the addition of 1 M NaOH on a pH-checker and centrifuged at 3000*g* ($r = 107$ mm) at 25 °C for 20 min. The supernatants were transferred into tubes and were ready for ELISA detection.

AOZ Assay. AOZ were determined by a simplified indirect competition ELISA (ic-ELISA) (*24*). The 96-well microtiter plates were coated by adding 100 μL of 3-[[4-carboxyphenyl]-methylene]amino-2-oxazolidinone (4-CPAOZ)−ovalbumin (OVA) conjugate (400 μg/L) in coating buffer to each well for 2 h at 4 °C. After washing 3 times with a washing buffer, the nonreacted binding sites on the plate were blocked for 1 h at 37 °C with 1% OVA (in PBS at pH 7.4). The plates were then washed 3 times with a washing buffer and stored in the dark at 4 °C. A total of 40 μL of matrix-matched calibration standard or samples were added into each well, which had previously been coated and blocked as described above, followed by the addition of 30 μL of antibody ($^1/_{60\,000}$ dilution in PBS) and 30 μL of horseradish peroxidas (HRP)-labeled anti-rabbit IgG ($^1/_{2000}$ dilution in PBS). After 40 min at 37 °C incubation, the plates were washed 5 times with a washing buffer. A total of 100 μL of 3,3′,5,5′-teramethylbenzidine (TMB) substrate solution was added to each well. The enzyme reaction was stopped after a 15 min incubation at room temperature by the addition of 50 μL of 2 M sulphuric acid. The absorbance values at 450 nm were measured by a microplate reader. The average optical density (OD) of B_0 wells, containing all components except the competitor, was taken to represent 100% activity. The mean absorbance of each standard was normalized against the mean absorbance of the zero standard (B/B_0). The analyte in a sample was calculated from the matrix-matched calibration curve.

The validation of the ELISA was similar to that described previously (*22*). The regression equations of the calibration curve of AOZ in swine muscle, liver, kidney, plasma, and urine are $y = -27.18x + 98.76$, $y = -31.14x + 114.88$, $y = -27.59x + 103.06$, $y = -26.04x + 119.73$, and $y = -20.42x + 67.28$ (where x represents B/B_0 and y represents log[AOZ (μg/kg and μg/L)]), respectively. The correlation coefficient of calibration curves was over 0.9932. The limit of quantification (LOQ) was 0.5 μg/kg for urine and 0.3 μg/kg for plasma and other tissues. Recoveries of AOZ in all tissues ranged from 65.9 to 98.0%, and the coefficients of variation (CVs) were less than 16.9%, at three incurred levels.

Data Analysis. Descriptive statistical parameters, such as mean, standard deviation (SD), and CV were calculated. Statistical analysis of

Table 1. AOZ Concentrations in Pig Tissues at Different Days after the Administration of Furazolidone

	concentration of AOZ in pigs (mean ± SD, μg/L and μg/kg)				
time (day)	plasma	muscle	liver	kidney	urine
0.5	1969.2 ± 202.6	691.0 ± 242.4	2169.7 ± 253.1	1011.8 ± 195.8	1260.3 ± 181.7
7	1413.7 ± 165.2	368.5 ± 90.8	1254.2 ± 174.4	563.3 ± 68.5	332.5 ± 44.6
21	106.1 ± 30.8	101.2 ± 31.5	169.3 ± 63.3	62.7 ± 16.7	96.5 ± 22.4
35	25.9 ± 6.4	17.6 ± 3.3	19.9 ± 4.2	4.5 ± 1.3	20.2 ± 5.0
56	5.1 ± 3.0	3.8 ± 1.0	7.3 ± 4.3	2.4 ± 0.5	3.7 ± 2.2
63	1.2 ± 0.5	0.6 ± 0.3	1.1 ± 0.2	0.6 ± 0.1	1.0 ± 0.3

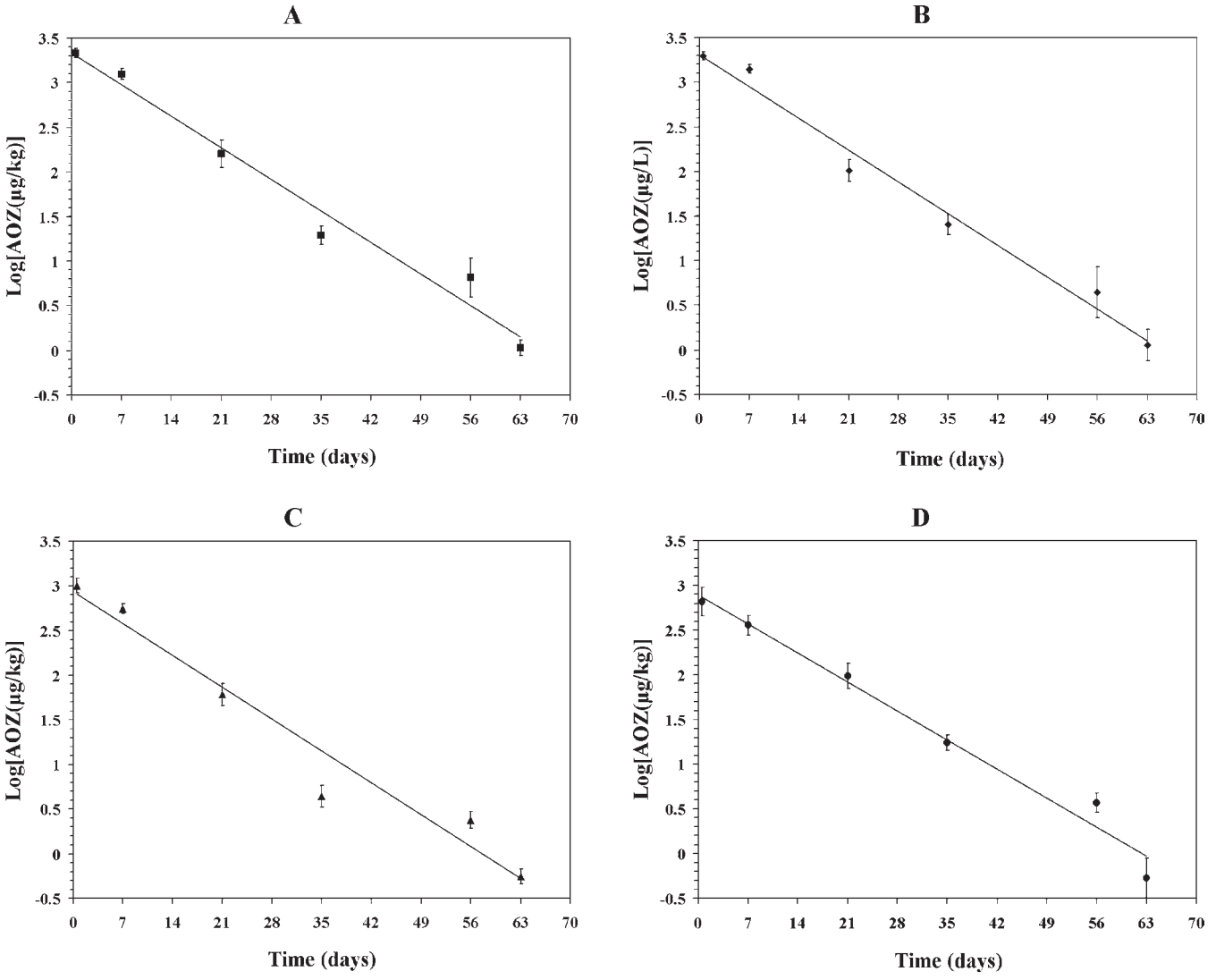

Figure 2. Elimination curve of AOZ in (A) liver, (B) plasma, (C) kidney, and (D) muscle of pigs fed furazolidone-medicated feed (400 mg/kg) for 7 days followed by a 9 week withdrawal period. Data are mean of four pigs per time point ± SD. Half-lives of AOZ calculated by the linear regression equation were 13.7, 13.6, 13.6, and 15.0 days for liver, plasma, kidney, and muscle, respectively. Linear equations for liver, plasma, kidney, and muscle are $y = -0.0505x + 3.3324$ ($r = 0.9880$), $y = -0.0509x + 3.3085$ ($r = 0.9917$), $y = -0.0509x + 2.9319$ ($r = 0.9777$), and $y = -0.0463x + 2.8931$ ($r = 0.9901$), respectively.

data and the correlation analysis between plasma/urine and tissues were performed using Microsoft Excel 2003 and SYSTAT SigmaPlot 11.0 (SPSS, Inc.).

The residue depletion profile of AOZ in tissues of pigs following withdrawal from the medicated diet was estimated by linear regression. The half-life ($t_{1/2}$) of AOZ in plasma and tissues during the elimination phase was calculated graphically by fitting a linear regression equation to the linear excretion curve of mean data obtained from day 0.5 to day 63, where the logarithm of the average AOZ concentration is plotted against time and where $t_{1/2} = \log 2/\alpha$ (with α being the slope of the fitted straight line).

Linear regression lines were developed using raw data in tissues and biological fluids (plasma and urine) to analysis the AOZ concentration correlations between tissues and biological fluids. A *t* test was carried out to statistically evaluate the correlation between biological fluid and edible tissues with 95% confidence intervals.

RESULTS

Elimination of AOZ from Tissues and Biological Fluids. The concentrations of AOZ for each time point were averaged, and the SD of each was calculated in **Table 1**. The highest concentration of AOZ in liver (2169.7 ± 253.1 μg/kg) was achieved at 12 h after the last dosing. At all time points, the highest concentrations were measured in the liver and the lowest concentrations were

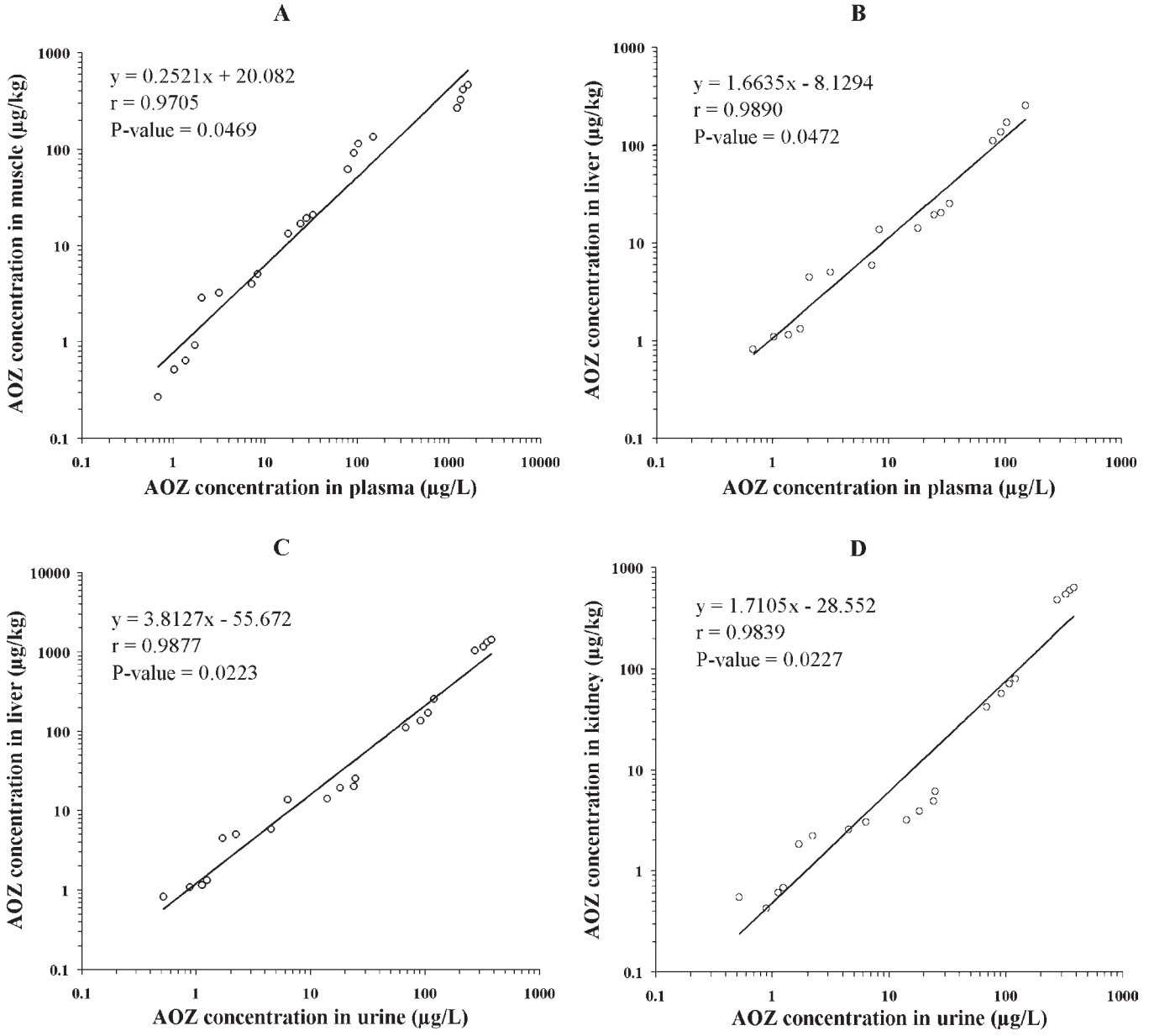

Figure 3. Correlation of AOZ concentrations in (A) plasma and muscle, (B) plasma and liver, (C) urine and liver, (D) urine and kidney.

measured in the muscle. AOZ could still be detected at 63 days in plasma, urine, and tissues, albeit at very low concentrations (0.37−1.72 μg/kg and μg/L). **Figure 2** illustrates the elimination of AOZ in liver, plasma, kidney, and muscle of pigs. Calculated elimination half-lives of AOZ by the linear regression equation in plasma, urine, liver, kidney, and muscle were 13.7, 14.7, 13.6, 13.6, and 15.0 days, respectively.

Correlations of the AOZ Concentration between Tissues and Biological Fluids. Computer-generated linear curves were produced to demonstrate the correlation of AOZ between different edible tissues and plasma and urine for the depletion time period of 7−63 or 21−63 days following the final administration of furazolidone (**Figure 3**). Good correlations were found for the plasma−muscle, plasma−liver, urine−liver, and urine−kidney combinations ($p < 0.05$) by developing linear regression lines. The linear correlation coefficients for plasma−muscle, plasma−liver, urine−liver, and urine−kidney were 0.9705, 0.9890, 0.9877, and 0.9839, respectively. The correlations of AOZ in plasma−liver were relatively superior to those in other tissues−biological fluids, judging by the correlation coefficients. AOZ concentrations in plasma were much closer to that in the liver, which contained the greatest amount of AOZ at all times.

DISCUSSION

The residue depletion results showed that, after the administration of furazolidone, a fraction of furazolidone was quickly metabolized to AOZ, which was extensively distributed in swine tissues and remained in the tissues for an extended period of time. With no withdrawal period, AOZ was most abundant in liver and plasma, followed by kidney and muscle. At 0−21 days, the concentration of AOZ in plasma, urine, and tissues decreased steadily, especially in liver and plasma. After that, the elimination of AOZ in plasma, urine, and tissues became very slow. At the 9 week withdrawal, AOZ was equally abundant in plasma, urine, muscle, kidney, and liver. These findings were consistent with the results on the elimination of AOZ by other studies. After a 9 week withdrawal period, AOZ concentrations from this study were in close agreement with those of Hoogenboom et al., McCracken et al., and Cooper et al., following 4, 7, and 6 week withdrawal periods, respectively (*8*, *17*, *18*). Similar depletion of AOZ was

also reported in carp (*24*), Nile tilapia (*27*), channel catfish (*28*), shrimp (*29*), and eel (*30*).

The longest depletion half-life of AOZ was observed in the muscle (15.0 days), and the shortest depletion half-life of AOZ was observed in the kidney (13.6 days). The differences in half-lives could possibly reflect the lower metabolic rate and slower cellular turnover in the muscle tissue when compared to the kidney. Half-life data for AOZ in this study were longer than reported by Cooper et al. (11.5, 6.9, and 7.3 days in the swine muscle, kidney, and liver, respectively) and McCracken et al. (9.1, 5.9, and 5.8 days in the swine muscle, kidney, and liver, respectively) (*8*, *18*). Hoogenboom et al. also reported that the longest AOZ half-life was determined in the swine muscle and the shortest AOZ half-life was determined in the kidney (*17*). The extended half-lives observed in this study may be caused by the longer withdrawal time (other researchers had a maximum withdrawal time of 7 weeks). In this study, the last withdrawal point was set at 9 weeks after the last administration of furazolidone. AOZ residue did exist in tissues, plasma, and urine at the final time point.

The concentrations of AOZ in plasma and urine are similar to those in tissues at the end of the elimination phase. On the basis of the good correlations of AOZ concentrations achieved, it is likely that, when AOZ was completely eliminated from each tissue, it could also not be detected in plasma and urine. If it can be detected in plasma and urine, it means that AOZ was still present in the edible tissues of pigs. That is the basis for using urine or plasma as an indicator for monitoring the illegal use of furazolidone (because there is no tolerance for AOZ residues in edible tissues). The decrease in the concentration of AOZ in urine can be monitored over time in a manner similar to the elimination of AOZ from the plasma, which reflects the reduction of the AOZ concentration in edible tissues over the same time period.

One research group suggested the use of swine retina as a matrix for the detection of furazolidone, because AOZ concentrates in the retina (*31*). However, in most countries, the removal of an eye for drug testing during the growth period would be unacceptable because of ethical concerns. Plasma and urine samples can be collected and analyzed multiple times at any time point from birth to slaughter of a pig. It seems that the use of plasma samples could provide a more reliable matrix for furazolidone monitoring when compared to urine. However, urine might be preferred over plasma for pre-slaughter testing of drug abuse because of the more convenient sample collection and preparation. Urine analysis can achieve the goal of no harm to the animal for the drug use monitoring.

A furazolidone tissue depletion and AOZ concentration correlations between edible tissues and biological fluids study in pigs were carried out using a simple, sensitive, and precise ic-ELISA. The data were analyzed and simulated to illustrate tissue residual behavior and investigate the correlations of the AOZ concentration in tissue−plasma and tissue−urine. With a strict statistical analysis, good correlations in the AOZ concentration were achieved for plasma−muscle, plasma−liver, urine−liver, and urine−kidney. On the basis of these correlations, plasma and urine could be used as an appropriate surrogate matrix for a fast, simple *in vivo* sample screening test under field conditions, such as in local farms and slaughterhouses. Analysis of AOZ concentrations from the urine/plasma samples may allow for detection of furazolidone residues in food-producing animals at any point of life. In comparison to LC−MS/MS, ELISA is probably more suitable for routine AOZ residue monitoring analyses under field conditions. The use of ic-ELISA would allow for more laboratories throughout the world to embark on a furazolidone-monitoring program. The application of this technology could lead to a significant improvement in the monitoring of the illegal use of furazolidone for food safety.

ABBREVIATIONS USED

AOZ, 3-amina-2-oxazolidinone; PAOZ, *N*-[phenzlidene]-3-amino-2-oxazolidinone; ic-ELISA, indirect competitive enzyme-linked immunosorbent assay; AVMA, American Veterinary Medical Association; BA, bezaldehyde; DMSO, dimethyl sulfoxide; OVA, ovalbumin; PBS, phosphate-buffered saline; LOQ, limit of quantification; 4-CPAOZ, 3-[[4-carboxyphenyl]-methylene]amino-2-oxazolidinone; HRP, horseradish peroxidase; TMB, 3,3′,5,5′-tetramethylbenzidine; SD, standard deviation; CV, coefficient of variation.

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LITERATURE CITED

(1) Ali, B. H. Pharmacological, therapeutic and toxicological properties of furazolidone: Some recent research. *Vet. Res. Commun.* **1999**, *23*, 343–360.

(2) Office for Official Publications of the European Communities. Commission Regulation (EC) 1442/95 of 26 June 1995 amending Annexes I, II, III and IV to Regulation (EEC) No 2377/90 laying down a community procedure for the establishment of maximum residue limits of veterinary medicinal products in foodstuffs of animal origin. *Off. J. Eur. Communities: Legis.* **1995**, *143*, 26–30.

(3) Ministry of Agriculture Bulletins. Ministry of Agriculture, People's Republic of China, **2002**; number 193, p 4.

(4) Cooper, K. M.; Elliott, C. T.; Kennedy, D. G. Detection of 3-amino-2-oxazolidinone (AOZ), a tissue-bound metabolite of the nitrofuran furazolidone, in prawn tissue by enzyme immunoassay. *Food Addit. Contam.* **2004**, *21*, 841–848.

(5) McCracken, R. J.; Blanchflower, W. J.; Rowan, C.; McCoy, M. A.; Kennedy, D. G. Determination of furazolidone in porcine tissue using thermospray liquid chromatography−mass spectrometry and a study of the pharmacokinetics and stability of its residues. *Analyst* **1995**, *120*, 2347–2351.

(6) McCracken, R. J.; Kennedy, D. G. Determination of the furazolidone metabolite, 3-amino-2-oxazolidinone, in porcine tissues using liquid chromatography−thermospray mass spectrometry and the occurrence of residues in pigs produced in Northern Ireland. *J. Chromatogr., B: Biomed. Sci. Appl.* **1997**, *691*, 87–94.

(7) McCracken, R. J.; McCoy, M. A.; Kennedy, D. G. The prevalence and possible causes of bound and extractable residues of the furazolidone metabolite 3-amino-2-oxazolidinone in porcine tissues. *Food Addit. Contam.* **1997**, *14*, 287–294.

(8) McCracken, R. J.; McCoy, M. A.; Kennedy, D. G. Furazolidone residues in pigs: Criteria to distinguish between treatment and contamination. *Food Addit. Contam.* **2000**, *17*, 75–82.

(9) Conneely, A.; Nugent, A.; O'Keeffe, M. Use of solid phase extraction for the isolation and clean-up of a derivatised furazolidone metabolite from animal tissues. *Analyst* **2002**, *127*, 705–709.

(10) Meijer, L. A.; Ceyssens, K. G.; de Jong, W. T.; de Greve, B. I. Correlation between tissue and plasma concentrations of oxytetracycline in veal calves. *J. Toxicol. Environ. Health* **1993**, *40*, 35–45.

(11) Li, T.; Qiao, G. L. Comparative plasma and tissue pharmacokinetics and drug residue profiles of different chemotherapeutants in fowls and rabbits. *J. Vet. Pharmacol. Ther.* **1995**, *18*, 260–273.

(12) Walker, C. C.; Thune, R. L.; Barker, S. A. Plasma/muscle ratios of sulfadimethoxine residues in channel catfish (*Ictalurus punctatus*). *J. Vet. Pharmacol. Ther.* **1995**, *18*, 306–310.

(13) Craigmill, A. L.; Holland, R. E.; Robinson, D.; Wetzlich, S.; Arndt, T. Serum pharmacokinetics of oxytetracycline in sheep and calves and tissue residue in sheep following a single intramuscular injection

of a long-act preparation. *J. Vet. Pharmacol. Ther.* **2000**, *23*, 345–352.
(14) Chiesa, O. A.; Von Bredow, J.; Heller, D.; Nochetto, C.; Smith, M.; Moulton, K.; Thomas, M. H. Use of tissue−fluid correlations to estimate gentamicin residues in kidney tissue of Holstein steers. *J. Vet. Pharmacol. Ther.* **2006**, *29*, 99–106.
(15) Chiesa, O. A.; Von Bredow, J.; Smith, M.; Heller, D.; Condon, R.; Thomas, M. H. Bovine kidney tissue/biological fluid correlation for penicillin. *J. Vet. Pharmacol. Ther.* **2006**, *29*, 299–306.
(16) Yang, B.; Huang, L.; Wang, Y.; Liu, Y.; Tao, Y.; Chen, D.; Liu, Z.; Fang, K.; Chen, Y.; Yuan, Z. Residue depletion and tissue−plasma correlation of methyl-3-quinoxaline-2-carboxylic acid after dietary administration of olaquindox in pigs. *J. Agric. Food Chem.* **2010**, *58*, 937–942.
(17) Hoogenboom, L. A. P.; Berghmans, M. C. J.; Polman, T. H. G.; Parker, R.; Shaw, I. C. Depletion of protein-bound furazolidone metabolites containing the 3-amino-2-oxazolidinone side chain from liver, kidney and muscle tissues from pigs. *Food Addit. Contam.* **1992**, *9*, 623–630.
(18) Cooper, K. M.; Mulder, P. P.; van Rhijn, J. A.; Kovacsics, L.; McCracken, R. J.; Young, P. B.; Kennedy, D. G. Depletion of four nitrofuran antibiotics and their tissue-bound metabolites in porcine tissues and determination using LC−MS/MS and HPLC−UV. *Food Addit. Contam.* **2005**, *22*, 406–414.
(19) Leitner, A.; Zöllner, P.; Lindner, W. Determination of the metabolites of nitrofuran antibiotics in animal tissue by high-performance liquid chromatography−tandem mass spectrometry. *J. Chromatogr., A* **2001**, *939*, 49–58.
(20) Conneely, A.; Nugent, A.; O'Keeffe, M.; Mulder, P. P. J.; van Rhijn, J. A.; Kovacsics, L.; Fodor, A.; McCracken, R. J.; Kennedy, D. G. Isolation of bound residues of nitrofuran drugs from tissue by solid-phase extraction with determination by liquid chromatography with UV and tandem mass spectrometric detection. *Anal. Chim. Acta* **2003**, *483*, 91–98.
(21) Diblikova, I.; Cooper, K. M.; Kennedy, D. G.; Franek, M. Monoclonal antibody-based ELISA for the quantification of nitrofuran metabolite 3-amino-2-oxazolidinone in tissues using a simplified sample preparation. *Anal. Chim. Acta* **2005**, *540*, 285–292.
(22) Chang, C.; Peng, D. P.; Wu, J. E.; Wang, Y. L.; Yuan, Z. H. Development of an indirect competitive ELISA for the detection of furazolidone marker residue in animal edible tissues. *J. Agric. Food Chem.* **2008**, *56*, 1525–1531.
(23) Cheng, C. C.; Hsieh, K. H.; Lei, Y. C.; Tai, Y. T.; Chang, T. H.; Sheu, S. Y.; Li, W. R.; Kuo, T. F. Development and residue screening of the furazolidone metabolite, 3-amino-2-oxazolidinone (AOZ), in cultured fish by an enzyme-linked immunosorbent assay. *J. Agric. Food Chem.* **2009**, *57*, 5687–5692.
(24) Liu, Y.; Peng, D.; Huang, L.; Wang, Y.; Chang, C.; Ihsan, A.; Tao, Y.; Yang, B.; Yuan, Z. Application of a modified enzyme-linked immunosorbent assay for 3-amino-2-oxazolidinone residue in aquatic animals. *Anal. Chim. Acta* **2010**, *664*, 151–157.
(25) National Office of Animal Health Limited. Compendium of data sheets for veterinary products 1992−93. Datapharm Publications Ltd., London, U.K., 1992; p 346.
(26) American Veterinary Medical Association (AVMA) Panel on Euthanasia. 2000 Report of the AVMA panel on euthanasia. *J. Am. Vet. Med. Assoc.* **2001**, *218*, 669.
(27) Xu, W. H.; Zhu, X. B.; Wang, X. T.; Deng, L. P.; Zhang, G. Residues of enrofloxacin, furazolidone and their metabolites in Nile tilapia (*Oreochromis niloticus*). *Aquaculture* **2005**, *254*, 1–8.
(28) Chu, P. S.; Lopez, M. I.; Abraham, A.; El Said, K. R.; Plakas, S. M. Residue depletion of nitrofuran drugs and their tissue-bound metabolites in channel catfish (*Ictalurus punctatus*) after oral dosing. *J. Agric. Food Chem.* **2008**, *56*, 8030–8034.
(29) Chu, P. S.; Lopez, M. I. Liquid chromatography−tandem mass spectrometry for the determination of protein-bound residues in shrimp dosed with nitrofurans. *J. Agric. Food Chem.* **2005**, *53*, 8934–8939.
(30) Krongpong, L.; Futami, K.; Katagiri, T.; Endo, M.; Maita, M. Application of ELISA-based kit for detecting AOZ and determining its clearance in eel tissues. *Fish. Sci.* **2008**, *74*, 1055–1061.
(31) Cooper, K. M.; Kennedy, D. G. Nitrofuran antibiotic metabolites detected at parts per million concentrations in retina of pigs—A new matrix for enhanced monitoring of nitrofuran abuse. *Analyst* **2005**, *130*, 466–468.

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