FULL PAPER *Public Health*

Determination of sulphachloropyrazine-diaveridine residues by high performance liquid chromatography in broiler edible tissues

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(Received 20 November 2014/Accepted 17 June 2015/Published online in J-STAGE 24 July 2015)

ABSTRACT. Diaveridine (DVD) is used in combination with sulphachloropyrazine (SPZ) as an effective antibacterial agent and antiprotozoal agent, respectively, in humans and animals. To gain a better understanding of the metabolism of SPZ and DVD in the food-producing animals, a high performance liquid chromatography (HPLC) method to determine and quantify sulphachloropyrazine (SPZ) and diaveridine (DVD) suspension residues from broilers is reported. Thirty healthy chickens were orally administered with sulphachloropyrazine-diaveridine (SPZ-DVD) suspension in water of 300 mg/*l* (SPZ) per day for seven successive days. Six chickens per day were slaughtered at 0, 1, 3, 5 and 7 days after the last administration. This procedure permitted SPZ and DVD to be separated from muscle tissue, liver, kidneys and skin with fat after extraction with acetonitrile and acetone under slightly acidic conditions. From the detected residuals in different tissues, we found that SPZ was quickly eliminated in liver and muscle, and slowly eliminated in kidney and skin with fat. DVD was quickly eliminated in liver and slowly eliminated in kidney. The withdrawal period of SPZ was 3.26, 3.72, 4.39 and 5.43 days in muscle, liver, kidney and skin with fat, respectively. The withdrawal period of DVD was 4.77, 4.94, 6.74 and 4.58 days in muscle, liver, kidney and skin with fat, respectively. Therefore, the suggested withdrawal period for SPZ-DVD suspension should be 7 days after dosing for seven successive days.
KEY WORDS: diaveridine, HPLC, residual metabolism, sulphachloropyrazine, withdrawal period

doi: 10.1292/jvms.14-0605; *J. Vet. Med. Sci.* 77(12): 1555–1563, 2015

Sulfonamides (SAs) are broad-spectrum antimicrobials inhibiting both Gram-positive and Gram-negative bacteria, as well as some protozoa, which are administered orally or mixed with animal feed of meat-producing animals to prevent and control a great variety of bacterial diseases [14, 19]. However, the widespread use of SAs in veterinary medicine may lead to SA accumulation in foodstuffs, which probably causes a variety of untoward reactions to humans after intake of animal-derived food products. Sulphachloropyrazine (SPZ) is synthetic bacteriostatic wide-spectrum antimicrobial, belonging to the group of SAs that are poorly absorbed from GI tract sulphonamides. SPZ is usually used to treat collibacteriosis, fowl cholera and cocidiosis infections in poultry in the daily dose of SCP 0.03% for 3–6 days [12, 17, 21]. Residues of SPZ may remain in edible animal tissues and then affect the human health, and this is the reason why many researchers focus on the development of a rapid, accurate, economical time and cost methods, for the determination of this antibiotic.

Diaveridine (DVD) is an antibacterial synergist which can interfere with bacterial nucleic acid synthesis [25]. DVD has broad-spectrum antibacterial activity against most Gram-negative and Gram-positive bacteria, including *Escherichia coli*, *Clostridium* spp., *Salmonella* spp., Staphylococcus aureus and Bacillus anthracis. DVD also has remarkable activity against coccidia. Therefore, it is widely used to prevent chicken coccocidiosis, fowl cholera and pullorum. DVD is rarely used by itself in the clinic; it is usually used in combination with sulfaguanidine and sulfamonomethoxine [4]. This drug combination can block the metabolism of folic acidin bacteria by two different mechanisms and even appears to have bactericidal effects [5]. DVD was found to be genetoxic in mammalian cells *in vitro* and *in vivo* [15]. Therefore, it is important to investigate the metabolism of SPZ and DVD in animals in order to assess the potential toxicity and food safety of the two compounds.

Many papers have been published concerning the assay method for veterinary drugs. Among them, bioassay and fluorometry, which are commonly used, present lack of sensitivity and specificity, while chromatographic methods are generally preferred for their greater selectivity and simplicity [6, 24]. A number of instrumental techniques have been introduced for the determination of veterinary residual *in vivo*, such as thin-layer chromatography, immunoenzymatic method, supercritical fluid chromatography, gas chromatography coupled with mass spectrometry and liquid chromatography tandem mass spectrometry [8, 20, 26]. However, the most prominent place among them is occupied by high performance liquid chromatography. The HPLC method is very effective in monitoring veterinary drugs, and that technique has been reported for the determination of SAs concentrations in various biological matrices [1, 10]. By this method, metabolism residues are determined either directly after the separation on a chromatographic column using a

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UV detector or indirectly by applying derivatization procedures followed by measurements on a fluorescent detector.

There is very poor information in the literature about tissue concentrations of SPZ-DVD suspension in any species, and therefore, it is hard to predict the withdrawal time in poultry after they medicated with SPZ-DVD suspension. Therefore, the present paper describes a rapid and specific procedure for HPLC determination of the SPZ and DVD content in broilers edible tissues after oral administration of DVD. Based on the results, we have proposed the pathways for DVD metabolism in chickens.

MATERIALS AND METHODS

Experimental material and reagents: Sulphachloropyrazine-diaveridine (SPZ-DVD) suspension, 20.0% SPZ with 4.0% DVD, was jointly developed by Nanjing Animal Husbandry Pharmaceutical Co., Ltd. and Jiangsu Beikang Pharmaceutical Co., Ltd. (Nanjing, China). The standard SPZ of chemical pure was purchased from China Institute of Veterinary Drugs Control (Beijing, China), and DVD of chemical pure was purchased from Augsburg (Frankfurt, Germany). The acetonitrile, methanol, n-Hexane and other chemicals (potassium dihydrogen phosphate, ortho-phosphoric acid 85% and dichloromethane) of HPLC grade were purchased from POCH chemical-company (Gliwice, Poland) at the highest purity available. The deionized water (18.2 M/cm) used for preparing all the aqueous solutions was obtained by the reverse osmosis method with Milli-Q-Plus 185 system (Millipore, Molsheim, France). This work was approved by the Ethic Committee of College of Veterinary Medicine, Yangzhou University.

Apparatus: Identification and quantification of analytes were carried out using a liquid chromatography apparatus (Agilent 1200, Chicago, IL, U.S.A.). It consisted of a solvent delivery pump (STAR9002), a manual injector and a variable wavelength UV-vis 9050 detector. The samples and standards were analyzed using a Kromasil C18 (250 × 4.6 mm) column (Kromasil; EKA Chemicals, Bohus, Sweden). An analytical balance (Sartorius BP 61S), Vortex (WH-1, Bio-mix, Warsaw, Poland), a centrifuge (TGL-16C, Anting, Shanghai, China), a Solid phase extraction (SPE) (Waters MCX, New York, NY, U.S.A.) and Milli Q Plus 185 system (Milipore-Waters) to produce deionized water were also employed.

Animals: All experiments were carried out on 30 healthy broilers of 40 days (d) of age of both sexes. Broilers were fed with SPZ-DVD suspension mixed in water for seven successive days, and then, they were divided into 5 experimental groups according to 5 different slaughter times (4 hr, 1, 3, 5 and 7 days, after withdraw time). There were also a group of broilers as a control, without feeding any drug. Before commencing the study, the broilers were marked with alphabetical code (broilers—1, 2, 3, 4 and 5) which shows each term of killing and taking of the samples.

The drug was mixed in drinking water, 300 mg/*l* SPZ water. The water was changed two times a day (at the morning, 1 hr before drug administration and at the evening). Then, fresh water without drug was replaced after the 7 days. All of the broilers ate and drank *ad libitum* throughout the period of this study. Control groups were given pure water by the same method of administration for 7 days continuously. Next, 6 broilers from a control group were humanitary slathered, and the marker tissues—muscles (100–200 g), liver (all), kidneys (all) and skin with fat (10–20 g) were taken for the recovery of SPZ and DVD.

Broilers were adapted to normal temperature (25°C), humidity (65%) and sunlight. The birds were freely fed standard food and water in metabolic cages for 1 week before the experiment. The chickens were not given food for 12 hr before the experiment started, but water was still given. Five chickens (3 females and 2 males) were administered a single dose of SPZ/DVD suspension in drinking water. The control chickens were housed and fed in the same way, but they were administered the same volume of 0.9% physiological saline.

The tissue samples were taken after 4 hr, 1, 3, 5 and 7 days of last drug application, in broilers. Matrices were cleaned of blood and stored at −20°C until the day of analysis by HPLC.

Chromatographic conditions: The sulfa drug under investigation is separated on a SEP column by isocratic elution using a mobile phase that consisted of acetonitrile-KH_2PO_4 (13:87; v/v) at a constant flow rate of 1.7 m*l*/min. The KH_2PO_4 was of pH 7.4, 0.01 M. The standards of sulphachloropyrazine and the tissue extracts were monitored at a wavelength of 270 nm. All analyses were performed at ambient temperature. The retention time of sulphachloropyrazine and DVD was about 10 min, similar to those in other studies. The chromatogram run time was 15 min.

Extraction procedure: Frozen tissue samples were thawed to room temperature prior to extraction. 2.00 g of test minced tissue (muscle, liver, kidney and skin with fat) was transferred into a test-tube and mixed with 15 m*l* of acetonitrile. After homogenization and centrifugation for 2 min at 15,000 rpm, the samples were sonicated for 5 min and centrifuged at 5,000 rpm for 10 min. The upper supernatant layer was transferred into a clean tube, for mixed 30 sec in Vortex with 15 m*l* of acetone and sonicated for 5 min. After third centrifugation for 3 min, the upper layer was combined and mixed with 5 m*l* of n-propanol. The mixture was dried in a rotary evaporator at 45°C.

Four m*l* of acetonitrile and 0.017 mol/*l* KH_2PO_4 (32/68) were added and next shaken for 5 min. The liquid was transferred to a glass tube after the residue was dissolved. The same amount of n-hexane was added to wash the liquid by vortexing for 2 min and centrifuging for 10 min at 4,000 rpm. The upper hexane layer was discarded. The lower liquid layer of 2 m*l* was added onto the MCX solid phase extraction column, which was balanced with 5 m*l* of methanol and 5 m*l* of hydrochloric acid in advance. After the liquid flow off, the column was washed with 2 m*l* of 0.1 mol/*l* hydrochloric acid, 2 m*l* of methanol and 10 m*l* of 10% ammoniation acetonitrile. The eluent was evaporated at 45°C. At last, the residue was dissolved with 1 m*l* of acetonitrile and 0.017 mol/*l* KH_2PO_4 (32/68). After filtered with 0.22 μm filter membrane, the extraction of SZP and DVD was injected into

the HPLC system. The extraction of SZP and DVD from the broiler tissues was performed according to Fig. 4.

Standards: A stock solution (10 mg/m*l*) of SPZ and DVD sodium salt was prepared by dissolving 10 mg of compound in 100 m*l* of methanol. Working solutions (100, 50, 10, 4, 1 and 0.04 mg/*l* for SPZ and 2, 1, 0.1 and 0.04 mg/*l* for DVD) were prepared by appropriate serial dilution of the stock solution with a mixture: and 0.017 mol/*l* KH2PO4 (32/68). These solutions were then injected in order to obtain the calibration curve.

Accuracy/recovery: The accuracy of the method was determined, by the recovery of drugs from the all control tissue samples of broilers (muscle, liver, kidney and skin with fat) spiked at 0.04, 1.0 and 10.0 μg/g for SPZ and 0.05, 1.0 and 2.0 μg/g for DVD. The extraction of SPZ and DVD was made in accordance to Scheme 1. 20 μl of samples were loaded onto HPLC for analysis.

RESULTS

Calibration, accuracy and recovery: Linearity was studied from matrix-matched calibration, spiking blank extracts at the five concentration levels. The regression equations were obtained using the 5-points concentration of standard. Table 1 shows that the good linearity was obtained for the 2 analytes with the correlation coefficient of >0.998. The calibration graph was obtained by plotting peak area against amount and was linear over the range 0.04–100 mg/*l* for SPZ and 0.04–4 mg/*l* for DVD. The equation for the calibration curve is y=64.597 × −0.4429, and the correlation coefficient equals 0.9992 for SPZ and y=28.185 × −0.3644 with correlation coefficient of 0.9999 for DND. The linear range experiments provided the necessary information to estimate the LOD and the LOQ limits based on the peak of lowest concentration in the linear range with a signal-to-noise ratio, S/N of 3.3 for limit of detection and 10 for limit of quantification. The limit of detection (LOD=3·Sxy/a, where Sxy is the standard deviation, and a is the slope of the calibration curve) is the lowest absolute concentration of analyte in a sample that can be detected but not necessarily quantified. The limit of quantification (LOQ=10·Sxy/a) is the lowest concentration of analyte in a sample that can be determined with acceptable precision and accuracy. LOD value is situated in the linearity range. The estimated values of LOD and LOQ were 0.02 mg/kg and 0.04 mg/kg for SPZ and 0.02 mg/kg and 0.04 mg/kg for DVD, respectively (Table 1).

Moreover, the accuracy of the procedure was obtained from relative standard deviation (RSD%) of areas calculated for three replicate injections of three increasing SPZ (0.04, 1.0 and 10.0 mg/*l*) and DVD (0.05, 1.0 and 2.0 mg/*l*) standard concentrations. The investigations were made on all tissues. The results of the assays presented in Table 2 suggest that the precision of the method, expressed as RSD, ranged from 0.7 to 7.0%. Table 2 summarizes the average recovery of SPZ and DVD in different tissues. Satisfactory results were obtained, and the recovery values were depending on tested tissues. The average recoveries were greater than 75.12 with coefficients of variation (CVs) between 0.72% and 6.98% for SPZ and greater than 74.46% with coefficients of variation (CVs) between 1.69 and 5.44% for DVD. Figures 1 and 2 show chromatograms obtained from broiler tissues containing SPZ and DVD, respectively. In order to verify the selectivity of the method, we analyzed all blank tissue samples from four tissues. No interference was detected in the region of interest where the analyte was eluted as is shown in the blank sample chromatograms. The stability of SPZ and DVD was determined in two different ways: in solvent (working solutions) and in tissue samples containing drugs. The working solutions were analyzed every week, and the instrumental responses were compared with peak areas obtained on the day of solution preparation. No degradation phenomenon was observed during storage of 4 months at 4°C.

Tissue residue depletion: Residues of SPZ and DVD in tissue specimens after oral administration of SPZ-DVD suspension (300 mg/*l* SPZ water, daily for 7 consecutive days) were determined. The tissue concentration time profiles are presented in Table 3 for kidney, liver, muscle and skin with fat. Mean tissue concentrations of SPZ and DVD, ranging from 0.58 μg/g to 5.62 μg/g and from 0.36 μg/g to 3.15 μg/g, respectively, were measured 4 hr after administration of the final dose of drugs (Table 3). During the observation time, the SPZ concentrations depleted much slower from the kidney and liver tissues than the muscle and skin with fat tissues. SPZ was detected in skin with fat (0.07μg/g), but not in other three tissues, 5 days after termination of drug treatment; SPZ can not be detected 7 days after cessation of drug in any tissue. In addition, a similar depletion rule was observed in DVD metabolism. DVD depleted much slower from the kidney and liver tissues than the muscle and skin with fat tissues. Marker residue concentrations of DVD were below the MRL (0.05 μg/g) in three tissue samples except in kidney, 5 days after the end of treatment. DVD can not be detected 7 days after cessation of drug in any tissue.

Withdrawal time estimation: The mean SPZ and DVD concentrations were below the LOQ at 7 days after cessation of medication in these four tissues (Table 3). The mean SPZ concentrations in muscle, kidney and liver were below the LOQ at 5 days after cessation of medication and below the LOD at 7 days after dosing. Linear regression analysis of the logarithmic transformed data can be considered for the calculation of the withdrawal periods by WT1.4 software. Using this approach, the withdrawal time was determined as the time when the one-sided 95% upper tolerance limit of the regression line with 95% confidence level was below the MRL [2]. Generally, when the majority of data from one slaughter point are below the LOD or LOQ, the whole time point should be excluded. Figure 3A and 3B illustrated a plot of withdrawal time calculation for SPZ and DVD in kidney after oral administration (300 mg/*l* SPZ water, daily for 7 consecutive days), respectively. Using this approach and considering the marker residue for the MRL, the withdrawal time for SPZ could be calculated for muscle, liver, kidney and skin with fat (300 mg/*l* SPZ water, daily for 7 consecutive days): 3.26, 3.72, 4.39 and 5.43 days, respectively. The withdraw time for DVD was calculated for muscle, liver, kidney and skin with fat by using the same approach: 4.77,

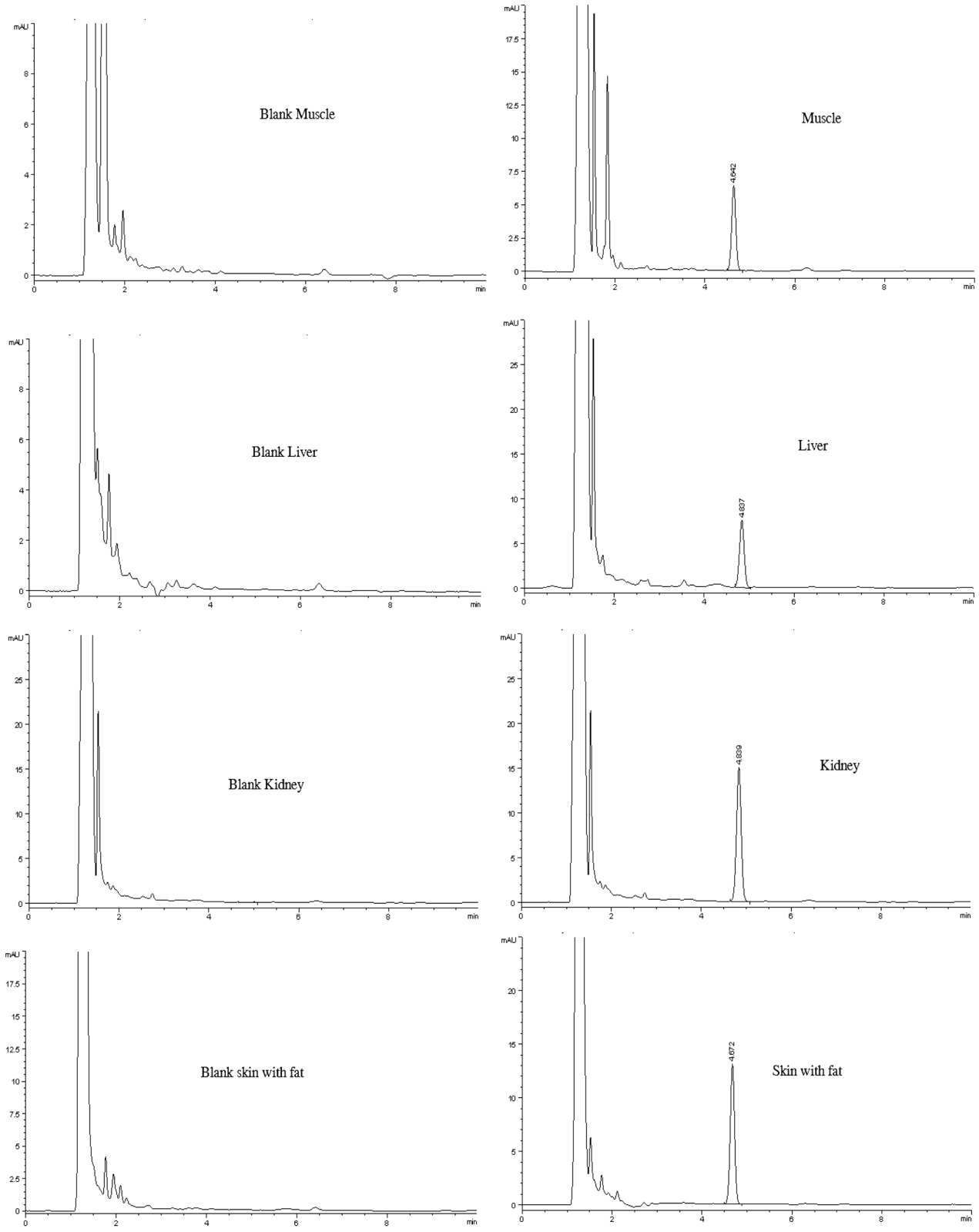

Fig. 1. Representative HPLC chromatograms obtained from broiler edible tissue samples (muscle, kidney, liver and skin with fat) spiked with 0.02 μg/g of SPZ and blank tissue samples.

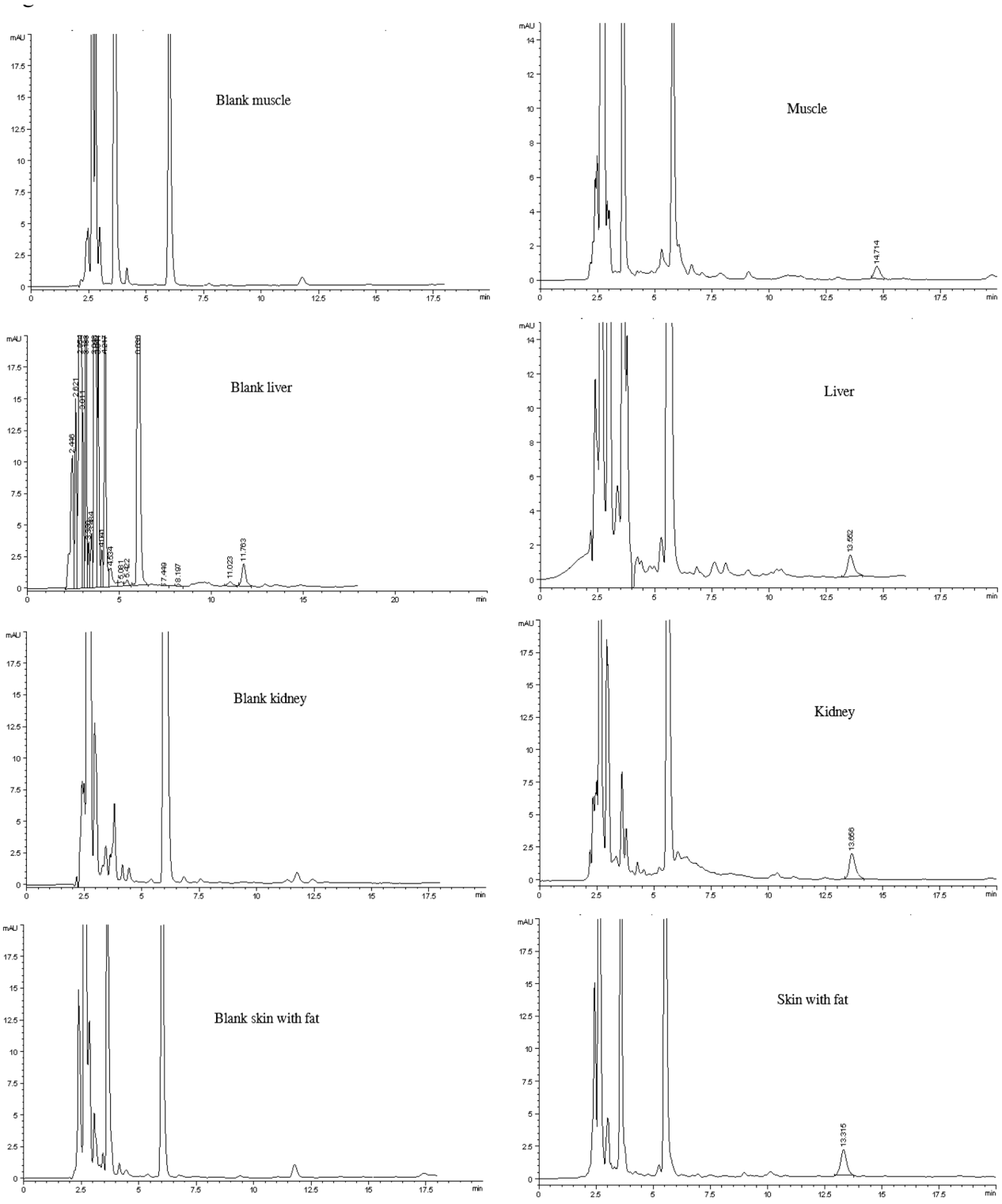

Fig. 2. Representative HPLC chromatograms obtained from broiler edible tissue samples (muscle, kidney, liver and skin with fat) spiked with 0.02 μg/g of DVD and blank tissue samples.

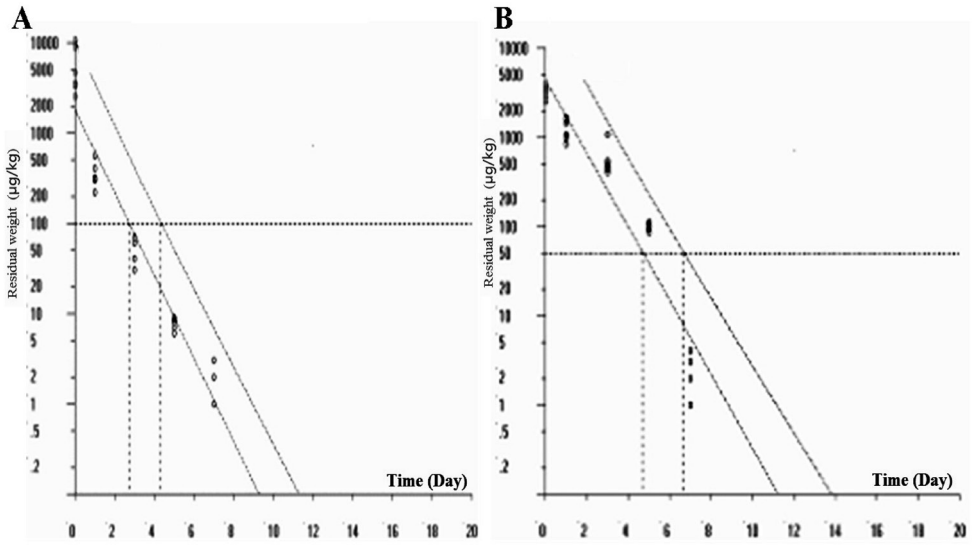

Fig. 3. Plot of the withdrawal time calculation for SPZ and DVD in broiler kidney at the time when the one-sided 95% upper tolerance limit is below the EU MRL for SPZ (A) and DVD (B) (500 μg kg-1) after oral administration of SPZ-DVD suspension (300 mg/*l* SPZ water, daily for 7 days).

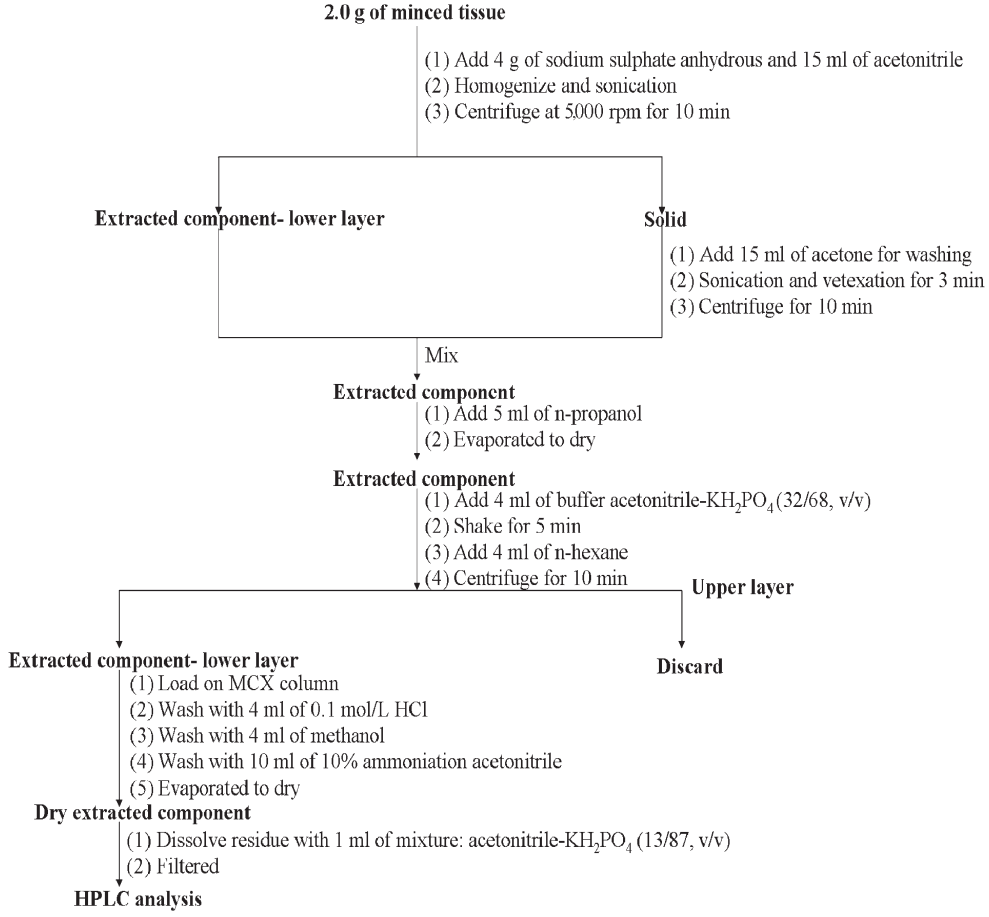

Fig. 4. Schematic diagram of solvent extraction.

Table 1. Analytical performance of the method

Analyte	Linearity range (mg/*l*)	Regression equation	γ	LOD (mg/*l*)	LOQ (mg/*l*)
SPZ	0.04–100	y=64.597 × −0.4429	0.9992	0.02	0.04
DVD	0.04–4	y=28.185 × −0.364	0.9999	0.02	0.04

Table 2. Determination of SPZ and DVD in different broiler edible tissues at three concentration levels

Drug	Tissue	Added 0.04 mg/*l*		Added 1.0 mg/*l*		Added 10.0 mg/*l*	
		Recovery (%)	RSD[a)] (%)	Recovery (%)	RSD[a)] (%)	Recovery (%)	RSD[a)] (%)
SPZ	Muscle	82.44	6.98	83.91	6.01	87.13	1.95
	Kidney	78.05	4.56	82.61	1.93	85.84	0.76
	Liver	75.12	2.04	78.37	1.34	79.75	0.72
	Skin with fat	80.00	3.18	81.52	0.89	86.34	0.98
		Added 0.05 mg/*l*		Added 1 mg/*l*		Added 2 mg/*l*	
		Recovery (%)	RSD[a] (%)	Recovery (%)	RSD[a)] (%)	Recovery (%)	RSD[a)] (%)
DVD	Muscle	76.05	3.71	93.02	6.91	94.03	2.36
	Kidney	79.60	4.54	92.56	5.20	92.85	4.54
	Liver	75.63	2.83	74.46	3.30	88.80	1.69
	Skin with fat	80.20	5.44	93.16	3.18	95.64	3.01

a) n=5.

Table 3. Precision date on the analysis of SPZ and DVD standard concentrations in different broiler edible tissues on five different days

Drug	Tissue	Mean concentration founded[a)] (μg/g ± SD)				
		4 hr	1 day	3 day	5 day	7 day
SPZ	Muscle	0.58 ± 0.38	0.10 ± 0.05	0.04 ± 0.02	ND	ND
	Kidney	5.62 ± 3.34	0.36 ± 0.12	0.04 ± 0.02	ND	ND
	Liver	1.35 ± 0.96	0.10 ± 0.03	0.02 ± 0.01	ND	ND
	Skin with fat	0.95 ± 0.54	0.34 ± 0.11	0.15 ± 0.05	0.07 ± 0.02	ND
DVD	Muscle	0.45 ± 0.17	0.20 ± 0.02	0.05 ± 0.03	0.02 ± 0.01	ND
	Kidney	3.15 ± 0.51	1.25 ± 0.33	0.57 ± 0.24	0.16 ± 0.02	ND
	Liver	2.22 ± 0.50	0.45 ± 0.15	0.13 ± 0.08	0.03 ± 0.01	ND
	Skin with fat	0.36 ± 0.14	0.18 ± 0.07	0.05 ± 0.02	0.03 ± 0.01	ND

a) n=5, ND: Not detectable.

4.94, 6.74 and 4.58 days, respectively. Therefore, by dosing SPZ-DVD suspension, a final withdrawal time of 7 days was determined.

DISCUSSION

Diaveridine (DVD) is a popular antibacterial synergist that is widely used in combination with SPZ. It has been reported to be genotoxic to mammalian cells, but more studies are required to clarify this [15, 18, 23]. Moreover, there is very little information on its pharmacokinetics and metabolic elimination. Prudent use of highly potent antimicrobials, such as DVD and SPZ, in veterinary medicine is strongly required to maintain the efficacy and safety of drugs for the future. Therefore, in order to gain a better understanding of the metabolism of DVD, we performed high-performance liquid chromatography (HPLC).

A critical aspect of drug residue analysis is the sample extraction purification steps required to isolate SPZ or DVD residues from broiler biological matrices. Sulphonamides and DVD have similar chemical and physicochemical properties. These compounds are not very soluble in non-polar solvents, but have good solubility in polar solvents. Traditionally, the extraction of sulphonamide from meat, milk and eggs has been done with organic solvents, such as: chloroform, methylene chloride, acetone, acetonitrile and ethyl acetate [1]. Clean-up methods reported in the literature use many different techniques: SPE cartridges filled with different stationary phases, e.g., silica gel, cationic exchanger, C18 and clean-up procedure with ultracentrifuge [7, 9]. Sample cleanup procedures also include liquid–liquid purification to reduce or eliminate interferences. Based on the extraction method for the determination of sulphonamides in animal tissues, acetonitrile was selected to precipitate proteins, and hexane was employed to extract out lipids [3, 10]. We tried another clean-up step i.e. ethanol, ethyl acetate, 5% solution of trichloroacetic acid and 1 M solution of hydrochloric acid, but this method permitted too many compounds that

interfered with the chromatography of the analyte. Results showed that deproteinization of the sample with acetonitrile followed by hexane washing of the aqueous acetonitrile extract could achieve significant purification. Subsequent extraction of SPZ was continued as a series of liquid–liquid partition clean-up procedures with the aid of dichloromethane and phosphate buffer. A double extraction improved better recovery of SPZ from animal tissues. Several spectroscopic techniques, such as UV absorption, FLD [22], DAD [1] or MS [10], are used for detection of different sulphonamides in LC. UV detection is often carried out at 270–280 nm, in some cases at 255 nm and 254 nm [11]. Based on the literature, 270 nm was selected as a wavelength for the separation of SCP in poultry tissue samples [16]. After optimization of the mobile phase parameters, the wavelength of detection and the flow rate, sulphachloropyrazine was detected with satisfactory recovery. Sulphachloropyrazine peak showed a good shape and no interferences with other peaks (impurities), which indicates a high selectivity and sensitivity of this method. The recovery of SPZ and DVD was higher than 70%, and the RSD was lower than 8%. This makes it valuable and adequate in many applications, particularly in veterinary medicine studies.

SPZ and DVD have a high potency and a broad spectrum of activity against a number of bacterial pathogens including the primary bacterial pathogens involved with chicken infections, such as *E. coli*, *Salmonella* spp.*, Pasteurella multocida, Pasteurella hemolytica, Haemophilus paragallinarum, Haemophilus gallinarum* and others. Tissue depletion of SPZ and DVD after daily oral administration of SPZ-DVD suspension (300 mg/*l* SPZ water for 7 consecutive days) was also determined. The dosage regimen of 300 mg/*l* SPZ water for 7 consecutive days was used, because the results presented here suggest from the integration of *in vitro* pharmacodynamics and *in vivo* pharmacokinetics that the drug should be administered orally at 20 mg/kg SPZ every 12 hr (or alternatively at 40 mg/kg every 24 hr), not only to guarantee clinical efficacy but also to minimize the selection and spread of resistant pathogens. The present work is the first to describe the residue tissue depletion of SPZ-DVD in edible chicken tissues using a validated HPLC method. SPZ and DVD concentrations in kidney, liver, muscle and skin with fat tissues were high initially, especially in kidney and liver and decreased over time. Concentrations of SPZ in tissues, except for skin with fat, were so much smaller than those detected for the parent drug SPZ-DVD. At total of 5 days after the last dose, the mean SPZ concentrations in all tissues were below the MRL (Table 3), and at total of 7 days after the last dose, the SPZ concentrations were not detectable. In a preliminary tissue distribution study of SPZ, the mean tissue concentration of SPZ also declined to mean values below MRL at 5 days after oral administration of 20 mg/kg for 3 consecutive days [11, 13]. This indicates that SPZ is not removed from the body at a slower rate when dosed at the higher level (40 mg/kg SPZ). It can be assumed that there are no dose-dependent differences in tissue drug distribution and elimination rate between those two doses. The study of DVD concentrations in broiler after 7 consecutive days also revealed a similar result.

There is a strict legislative framework controlling the use of antimicrobial substances, with the aim of minimizing the risk to human health associated with consumption of their residues. Therefore, to ensure human food safety, the European Union (EU) has set the tolerance level for these compounds as the maximum residue limit (MRL). The MRL in all food producing species including chicken was fixed for SPZ or DVD at 0.1μg/g, while 0.05 μg/g in Japan. According to the depletion regimen, a withdraw time of SPZ and DVD was calculated by using WT1.4 software with MRL at 0.05 μg/g. The withdraw times for SPZ in muscle, liver, kidney and skin with fat were 3.26, 3.72, 4.39 and 5.43 days, respectively; and 4.77, 4.94, 6.74 and 4.58 days, respectively, for DVD. Based on the data, the suggested withdraw time for SPZ-DVD suspension administration for 7 consecutive days was 7 days.

Our study provides data for a more prudent use of SPZ and DVD in broiler, suggesting a possible rational dosing and a withdrawal time after treatment to guarantee safety in foods for the consumers.

ACKNOWLEDGMENT. The work was financially supported by the Priority Academic Program Development of Jiangsu Higher Education Institutions and the Sponsored by Qinlan Project of Jiangsu (2012).

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