

Ceftiofur Distribution in Plasma and Tissues Following Subcutaneously Administration in Ducks

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ABSTRACT. A study was performed to determine the residues in blood and edible tissues of healthy ducks (25 days old, mean body weight 1.0 ± 0.13 kg) after subcutaneous administration of ceftiofur sodium at a dose rate of 2 mg/kg body weight (Group I) and 4 mg/kg body weight (Group II). Blood, muscle, liver, kidney, and fat samples were collected from all of ducks on the 1st, 2nd, 3rd, 4th, and 5th day after treatment of drug, and ceftiofur was analyzed with a high-performance liquid chromatography (HPLC) assay with results reported as ceftiofur-free acid equivalent (CFAE). To study the spiked recovery, blank plasma and tissues were spiked with two different concentrations of ceftiofur sodium (0.1, 0.5 $\mu\text{g/g}$). Average recovery values for all samples ranged from 70.3 to 87.3%. In the group I, desfuroylceftiofur acetamide (DCA) was not detected in all of plasma, muscle, liver, and fat tissues on the 1st day after treatment. But, kidney samples on the 1st day were detected DCA (0.059 ± 0.01 $\mu\text{g CFAE/g tissue}$). On the 2nd day of post-treatment, the concentrations of DCA in all tissues were lower than the detection limit, 0.05 $\mu\text{g CFAE/g tissue}$. In the group II on the 1st day after treatment, the concentration of DCA was 0.124 ± 0.06 $\mu\text{g CFAE/g tissue}$, 0.103 ± 0.03 $\mu\text{g CFAE/g tissue}$, and 0.071 ± 0.010 $\mu\text{g CFAE/g tissue}$ in plasma, kidney, and muscle samples, respectively. On the 2nd day after treatment of ceftiofur, the concentrations of DCA in all tissues were lower than 0.05 $\mu\text{g CFAE/g tissue}$. According to our results, the concentrations of DCA on the 1st day after treatment with 2 mg/kg body weight were below 0.05 $\mu\text{g CFAE/g tissue}$ equivalent in all tissues except for kidney. On the 2nd day after administration at the dose of 4 mg/kg body weight, no DCA was also detected in all of the tissues although DCA was detected in all samples on the 1st day.

KEY WORDS: ceftiofur, duck, HPLC.

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Ceftiofur is a third generation cephalosporin with the broad spectrum bactericidal activity against a variety of gram positive, gram negative and anaerobic pathogens [2]. Also, ceftiofur is known to be resistant to β -lactamase enzymes [17].

Due to the safety of residue in edible tissues and milk, ceftiofur was approved for use in the United States in cattle, pigs, poultry, horses, dogs, goat, and sheep [5–7, 11]. Ceftiofur has been world-widely used for cattle to treat bovine respiratory disease associated with *Pasteurella hemolytica*, *Pasteurella multocida*, and *Haemophilus somnus*, for swine to treat pleuropneumonia caused by *Actinobacillus pleuropneumonia*, and for chickens to treat fowl typhus and diarrhea associated with *Salmonella* Garinarium and *Escherichia coli* [10, 18].

The pharmacokinetics of ceftiofur in various species was reviewed by Brown *et al.* [4], and additional researches for ceftiofur have been reported for cattle, horses, dogs, goat, and sheep [3, 6–9, 12, 16, 19].

Although ceftiofur sodium has been widely used for the prevention and treatment of diseases on the domestic animals including ducks, there are rarely experimental data for ceftiofur residue on duck tissues during the withdrawal period.

The purpose of this study was to determine the distribution of ceftiofur in tissues of farming ducks after single subcutaneously doses of 2.0 mg ceftiofur sodium per kg body weight, the recommended therapeutic dose, and 4.0 mg ceftiofur sodium per kg body weight. As some farmers have sometimes used over-dose of antibiotics in a clinical setting, this study was used a dosage of 4.0 mg ceftiofur sodium per kg body weight as over-dose to identify the duck tissue residues of ceftiofur sodium.

One hundred healthy White Pekin ducks (*Anas platyrynchos*) with an average weight of 1.0 kg were assigned to two groups; a group (Group I) consisting of fifty ducks treated with a subcutaneous injection of ceftiofur sodium at the dose of 2 mg per kg body weight and another group (Group II) consisting of the other fifty ducks treated with a subcutaneous injection of ceftiofur sodium 4 mg per kg body weight. Accent® Plus (1 g, ceftiofur sodium) was supplied by the LG Life Sciences Ltd. (Seoul, Korea). The ducks were housed in cages equipped with feeders and waterers. The ducks were purchased from Sancheong Duck Farm (Sancheong, Korea). Temperature in the room was thermostatically controlled by ventilation fans. Fluorescent bulbs provided 24 hr of light. Ducks had unrestricted access to food and water. Ten of blood, muscle, liver, kidney, and fat samples were collected from each group on the 1st, 2nd, 3rd, 4th, and 5th days after administration of ceftiofur sodium. Blood samples were collected in heparinized tubes and centrifuged at $3,500 \times g$ for 10 min at room temperature to col-

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lect plasma. Aliquots of 1.5–2 ml plasma were stored at -20°C until analysis. Also, muscle, liver, kidney, and fat samples were stored at -20°C until analysis.

Analytical methods of ceftiofur have extracted derivatized analyte from plasma and tissue using solid-phase extraction clean-up steps followed by an established, validated HPLC analysis with results reported as ceftiofur-free acid equivalent (CFAE) [1]. This method was modified and validated for the detection of ceftiofur sodium in plasma, muscle, kidney, liver, and fat samples. In short, 1.0 ml plasma was mixed with 5 ml of extracting solution (0.4% (w/v) dithioerythritol in borate buffer pH 9) and incubated at 50°C for 15 min with intermittent mixing at every 5 min interval. Following reduction, 5 ml 14% (w/v) iodoacetamide in phosphate buffer was added, mixed and incubation was continued at room temperature for 30 min in the dark. The 10 g tissue (muscle, liver, kidney, and fat) was mixed with 140 ml of extracting solution. After homogenization, one 15 ml aliquot (1 g tissue equivalent) of homogenate from each blender bowl was transferred to a 50 ml centrifuge tube and incubation was carried out as described for plasma. Suspensions were acidified at $\text{pH } 2.5 \pm 0.1$ by addition of phosphoric acid. After centrifugation at $3500 \times g$ for 25 min at 4°C , supernatants were transferred onto preconditioned C_{18} solid-phase extraction (SPE) cartridges (1 g, Bond Elut, Varian). The C_{18} SPE cartridges were preconditioned with 4 ml of methanol followed by 5 ml of phosphate buffer. The supernatant was charged onto the cartridges using gravity feed. The cartridges were washed with 5 ml of phosphate buffer, followed by 3 ml of 0.01 M sodium hydroxide. New collection tubes were put in the manifold. A 3 ml mixture of acetonitrile and water at 15:85 (v/v) was added and cartridges were allowed to drain by gravity feed. Then, vacuum was used to drain the remaining solution in the cartridge. The collection tubes were removed and 15 ml of water were added to each tube to give a total volume of 18 ml. The elutes were transferred to preconditioned SAX-SPE cartridges (200 mg, Bond Elut, Varian), allowed to drain by gravity feed (vacuum was used to charge the fat samples at an approximate rate of 1 drop/sec) and washed with 1 ml water. The elutes were transferred to preconditioned SAX-SPE cartridges (500 mg, Bond Elut, Varian). New collection tubes were placed in the manifold and the cartridge contents were eluted with 2.5 ml mixture of acetonitrile and 5% acetic acid in water (5:95, v/v). The SAX-SPE cartridges were preconditioned with 2 ml of methanol, followed by 2 ml of SAX prewash solution and two times with 1 ml of water. At this point, this elute was analyzed by HPLC for the plasma and fat samples and the corresponding calibration standards for plasma and fat.

To all other collection tubes, 10 ml of water were added (for a total of 12.5 ml) and the tubes were mixed well. The SCX cartridges were preconditioned with 1 ml of methanol followed by 2 ml of SCX prewash solution (methanol-0.1 M calcium chloride, 25:75, v/v) and twice with 1 ml of water. The samples were transferred to the SCX cartridges and allowed to drain by gravity feed. The cartridges were then

washed with 1 ml water. New collection tubes were placed on the manifold. Muscle, liver and kidney samples and the corresponding standards were eluted with 2.5 ml mixture of acetonitrile and 0.1 M sodium chloride (5:95, v/v).

Samples were analyzed on a Hewlett-Packard 1100 series LC system (ALS, DAD). A Waters Spherisorb C_{18} column (4.6×200 mm, $5 \mu\text{m}$) was used. The volume of injection was $20 \mu\text{l}$. Elution of analytes was performed using a binary linear gradient using 0.1% trifluoroacetic acid (TFA) in water (mobile phase A) and 0.1% TFA in acetonitrile (mobile phase B). The flow-rate was 1.0 ml/min. The column was washed with 50% mobile phase B for 15 min (1.5 ml/min) and equilibrated with starting conditions (0% mobile phase B) for 20 min before the next injection. The HPLC-elute was monitored at 266 nm.

Concentrations of CFAE in tissues were calculated from the standard curves constructed by plotting the area of desfuoylceftiofur acetamide (DCA) against the working standard concentrations of ceftiofur sodium (0.025, 0.05, 0.1, 0.5, and $1.0 \mu\text{g/g}$). All data were presented as mean \pm SD. The recovery of ceftiofur was assessed in determinations at spiked five tissue samples. The responses from the spiked samples were compared with those from the negative control sample. The limit of detection (LOD) was established with injection of tissue blanks fortified with the internal standard and measuring the baseline noise at the time of retention of DCA peak. The mean baseline noise at DCA retention time plus three standard deviations was defined as the detection limit. The mean baseline noise plus ten standard deviations was defined as the limit of quantification (LOQ).

The standard calibration curve for CFAE was constructed to determine the detection limit. As shown Fig. 1, the detection limit of DCA was lower than $0.05 \mu\text{g CFAE/g tissue}$.

The standard curve for CFAE showed linear regression between 0.05 to $0.5 \mu\text{g/g}$ ($R^2=0.995$). Recovery values of 0.1 and $0.5 \mu\text{g/g}$ of ceftiofur spiked into non-treated plasma and tissue samples was shown in Table 1. Average recovery

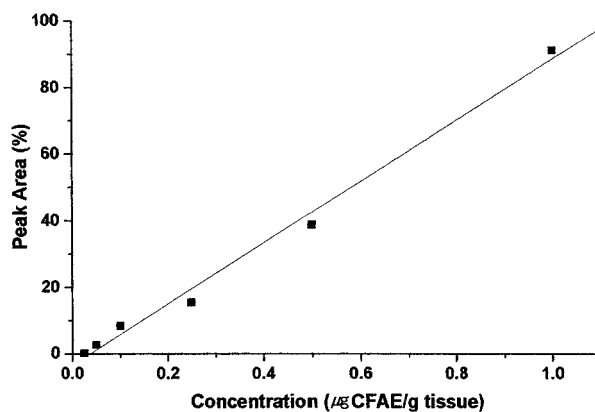


Fig. 1. Calibration curve for ceftiofur-free acid equivalent (CFAE) calculated from peak areas of desfuoylceftiofur acetamide (DCA) of ceftiofur standard. The detection limit of DCA was calculated at $0.05 \mu\text{g CFAE/g tissue}$ equivalent.

Table 1. Recoveries of ceftiofur-free acid equivalent in duck tissue samples spiked to ceftiofur

Tissue	No. of Samples	Spiked Concentration ($\mu\text{g/g}$)	Recovery (%)	
			Range	Mean \pm SD
Plasma	5	0.1	73.2–80.5	76.3 \pm 1.23
	5	0.5	78.6–87.6	83.4 \pm 14.7
Muscle	5	0.1	83.2–94.5	87.3 \pm 5.15
	5	0.5	76.6–84.1	80.2 \pm 3.27
Fat	5	0.1	68.2–71.3	70.3 \pm 0.92
	5	0.5	68.5–73.8	71.4 \pm 2.95
Liver	5	0.1	75.6–86.3	81.2 \pm 4.15
	5	0.5	72.4–78.9	75.7 \pm 2.73
Kidney	5	0.1	79.5–89.2	84.2 \pm 4.41
	5	0.5	80.2–87.1	83.3 \pm 3.10

values ranged from 76.3 to 83.4%, 80.2 to 87.3%, 70.3 to 71.4%, 75.7 to 81.2%, and 83.3 to 84.2% for plasma, muscle, fat, liver and kidney, respectively.

Figure 2 shows chromatograms of ceftiofur standard and duck tissue samples spiked with ceftiofur showing DCA. According to Fig. 2, the DCA peak of ceftiofur standard was 18.3 min and that of fortified tissue samples was showed at 10.5 min. The limits of detection and quantification were 0.05 and 0.1 $\mu\text{g/g}$, respectively.

The analytical results of ceftiofur in duck tissue samples are shown in Table 2.

In the group I, which was treated with ceftiofur 2 mg per kg body weight, ceftiofur was only detected in kidney samples ($0.059 \pm 0.01 \mu\text{g CFCA/g tissue}$) on the 1st day after administration. In the case of group II, which was administered with ceftiofur 4 mg/kg body weight, ceftiofur was detected in all of the samples and the concentration of CFCA was ranged from 0.066 to 0.124 $\mu\text{g/g}$ on the 1st day after treatment. After the 2nd day of treatment, the concentrations of DCA in all tissue samples of group I and group II were lower than 0.05 $\mu\text{g CFCA/g tissue}$, the limit of detection.

The HPLC procedure applied in this study for the detection of ceftiofur in duck tissue samples had 0.05 $\mu\text{g/g}$ detection limit and 0.2 $\mu\text{g/g}$ quantification limit. All recoveries of tissue samples were more than 70.3% of the spiked value. The calibration curve of ceftiofur showed good linearity (concentration range, 0.05 to 0.5 $\mu\text{g/g}$; $R^2=0.995$). McNeilly *et al.* [15] studied the determination of ceftiofur in bovine milk by HPLC, and investigated recovery rates after spiked at the dose of 0.025, 0.5, and 0.1 $\mu\text{g/g}$ of ceftiofur in bovine milk. The range of recovery rate for ceftiofur was 86.1–92.0%, and the LOD and LOQ were estimated to be 4 and 7 $\mu\text{g/kg}$, respectively. Lambert and Lena [14] carried out determination of cephalosporins in raw bovine milk by HPLC, and examined recovery rates of ceftiofur. In the concentration range 0.02–0.2 $\mu\text{g/g}$, the mean recoveries were 84–88% for ceftiofur. And the LOD and LOQ were 7 and 9 $\mu\text{g/kg}$, respectively. In the research by Beconi-Barker *et al.*

[1], the calibration curve for ceftiofur was linear for the entire calibration range of 0.05–10 $\mu\text{g/g}$ swine muscle, and recovery values for ceftiofur ranged from 70.4 to 85.0%, 74.7 to 88.0%, 88.3 to 94.9%, and 85.4 to 89.4% for muscle, kidney, liver and fat, respectively. Jacobson *et al.* [13] studied the determination of ceftiofur in bovine plasma by HPLC-DAD, and investigated the calibration curve and recovery rate after spiked at the concentration of 5 $\mu\text{g/g}$ ceftiofur in bovine plasma. The calibration curve for CFAE was linear over the range 0.4–40 $\mu\text{g/g}$ with $R^2=0.995$, and the recovery was 99.9%. The LOD and LOQ were 400 and 150 $\mu\text{g/kg}$, respectively.

In our study, the recovery rates of ceftiofur and the LOD and LOQ were lower than results of the research carried out McNeilly *et al.* [15] and Lambert and Lena [14]. And the recovery rates of ceftiofur were similar to those in the research carried out Beconi-Barker *et al.* [1], and lower than that in the study by Jacobson *et al.* [13]. The calibration curve in this study was linear over the range 0.05–0.5 $\mu\text{g/g}$ with $R^2=0.995$, and the result was similar to that in the research carried out Jacobson *et al.* [13].

While the recovery rate of the research carried out Jacobson *et al.* [13] was higher than those of other studies including our research, the research by Jacobson *et al.* [13] could be performed without the need for solid-phase extraction, and then the assay was less sensitive than other assays. It was assumed that the different results of recovery rates were depended on experimental conditions, kinds of sample, and methods of sample extraction.

Tell *et al.* [18] investigated pharmacokinetics of ceftiofur sodium in domestic chicks, cockatiels, and Amazon parrots after subcutaneous (chicks) and intramuscular (cockatiels and Amazon parrot) dosing. At 8 hr after a single subcutaneous injection at the dose of 0.16 mg/chick, the concentration of ceftiofur in plasma of chicks was 0.58 $\mu\text{g/g}$. Following intramuscular administration at the dose of 10 mg/kg body weight, ceftiofur was detected 0.09 $\mu\text{g/g}$ in the plasma of cockatiels by 12 hr post-treatment. In the Amazon parrots, ceftiofur was declined to 0.27 $\mu\text{g/g}$ in the

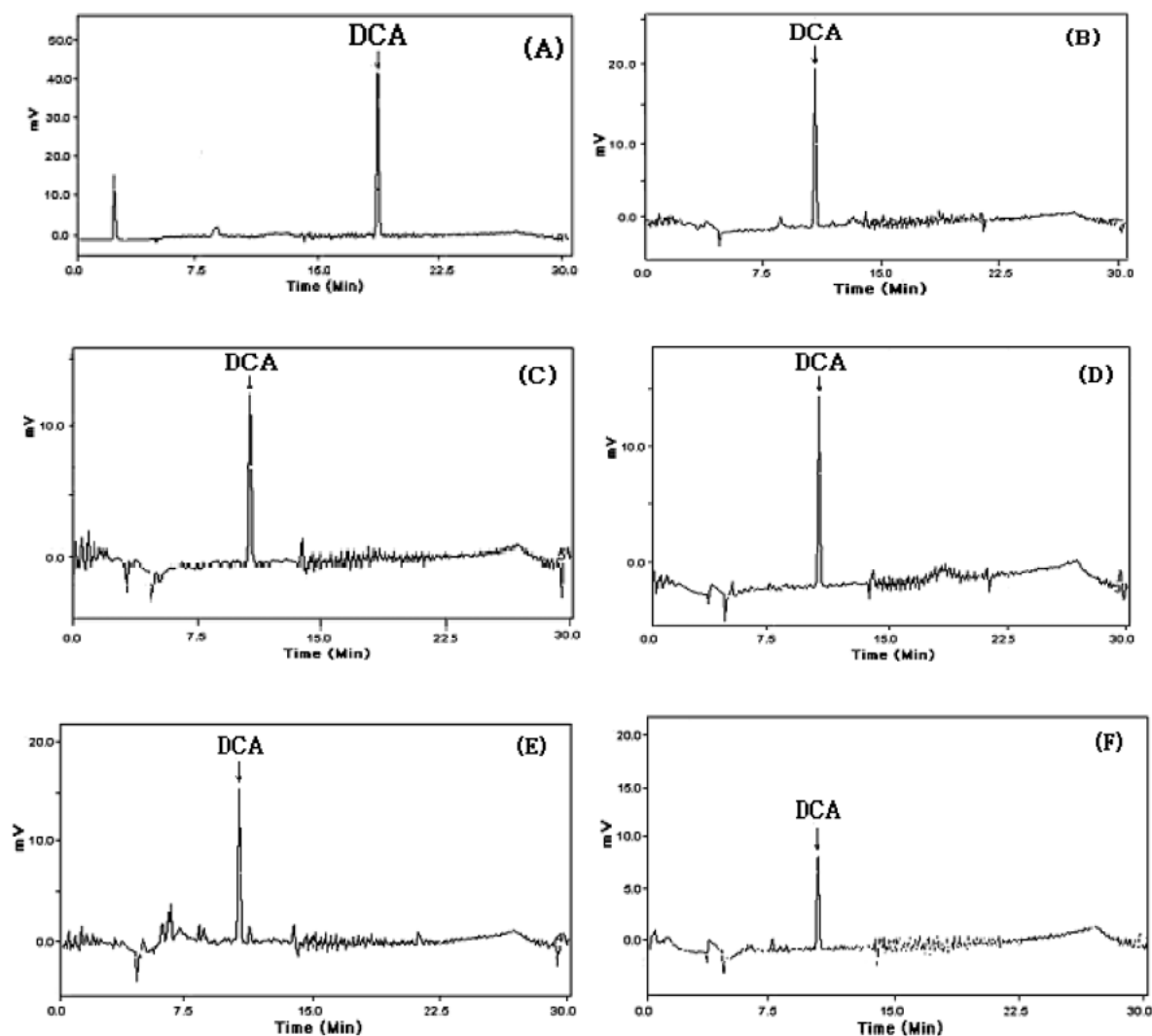


Fig. 2. Representative chromatograms obtained from various duck tissue samples spiked to a level of $10 \mu\text{g}$ CFAE/g tissue. (A), ceftiofur standard; (B), plasma; (C), muscle tissue; (D), fat tissue; (E), liver tissue; (F), kidney tissue.

Table 2. Depletion profiles of ceftiofur-free acid equivalent ($\mu\text{g/g}$) in duck tissues during withdrawal period following subcutaneous administration of ceftiofur

Tissue	No of Sample	Group I ^{a)}					Group II				
		1 ^{b)}	2	3	4	5	1	2	3	4	5
Plasma	10	ND	ND	ND	ND	ND	0.124 ± 0.06	ND	ND	ND	ND
Fat	10	ND	ND	ND	ND	ND	0.072 ± 0.02	ND	ND	ND	ND
Muscle	10	ND	ND	ND	ND	ND	0.071 ± 0.01	ND	ND	ND	ND
Liver	10	ND	ND	ND	ND	ND	0.066 ± 0.01	ND	ND	ND	ND
Kidney	10	0.059 ± 0.01	ND	ND	ND	ND	0.103 ± 0.03	ND	ND	ND	ND

ND, Not detected.

a) Group I and group II was administered with ceftiofur sodium 2 and 4 mg/kg body weight, respectively.

b) The day after administration of ceftiofur sodium.

plasma by 24 hr post intramuscular administration at the concentration of 10 mg/kg body weight.

Brown *et al.* [3] carried out a comparative study of plasma pharmacokinetics of ceftiofur sodium and ceftiofur hydrochloride in pigs after a single intramuscular injection at the dose of 3 mg or 5 mg/body weight, and analyzed these in the plasma with HPLC method. The analytical results showed that the concentration of ceftiofur sodium at 72 hr after the 3 mg injection was 0.27 $\mu\text{g/g}$, and that at 96 hr after the 5 mg injection was 0.224 $\mu\text{g/g}$.

Jaglan *et al.* [12] investigated concentration of ceftiofur metabolites in the plasma and lungs of horses following intramuscular treatment at the concentration of 2.2 mg/kg body weight. At 24 hr after administration, the concentration of ceftiofur in plasma was 0.17 $\mu\text{g/g}$. In red deer, Drew *et al.* [8] studied pharmacokinetics of ceftiofur after intramuscular administration at the dose of ceftiofur sodium 150 mg/kg body weight. Ceftiofur concentrations of all plasma samples were below 0.2 $\mu\text{g/g}$ at 24 hr after administration.

With the consideration of species, the dosage and the route administered, the plasma and tissue residue concentrations of DCA in our study were similar or little lower than those of studies previously described.

According to our results, the concentrations of DCA on the 1st day after treatment with ceftiofur sodium 2 mg/kg body weight were below 0.05 μg CFAE/g tissue in all tissues of duck except for kidney. On the 2nd days after administration at the dose of 4 mg/kg body weight, no DCA was also detected in all tissues of duck although DCA was detected in all samples on the 1st day.

Therefore, it is suggested that after subcutaneous administration of ceftiofur at the dose of 2 and 4 mg/kg body weight, the withdrawal period of ceftiofur in duck is established within 2 days.

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