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Residues of Ceftiofur Sodium in Rabbit Tissues

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Abstract

This study was designed to determine ceftiofur sodium residues of different rabbit tissues after intramuscular injection at a dose of 2.2 mg/kg BW. A total of twenty-four healthy male New Zealand White rabbits were divided into two groups; the first group (n = 21) was injected with ceftiofur for five successive days, while the second group (n = 3) untreated (control). Liver, kidney, pectoral and thigh muscles, spleen, heart, blood and lung from each rabbit were collected at the 1st, 3rd, 5th, 7th, 9th, 15th and 21st day post ceftiofur sodium treatment. Tissues were extracted and ceftiofur residues were analyzed using high-performance liquid chromatography (HPLC). Ceftiofur remained within the detectable level till the 5th day in most of the investigated tissues (liver, kidney, lung, heart, pectoral and thigh muscles) and serum, but still detected till the 7th and 9th day post treatment in lung and kidney, respectively. It can be concluded that rabbit muscles and livers could be consumed safely at the 3rd day post treatment with that dose, while, rabbit kidneys could be consumed safely at the 1st day post treatment with that dose without any hazards on consumers because the residual level is below the recommended MRL.

Keywords: Ceftiofur, Residues, Rabbits, HPLC.

Introduction

Over the last years, the food production system was changed to a large-scale production. Consequently, the administration of drugs to treat and/or prevent the spread of infections in food producing animals was increased [1]. Therefore, the presence of drugs residues in different tissues of treated animals may increase the risk of antibiotic resistance or other adverse effects on people consuming meat and/or animal by-products [2]. WHO and FAO established maximal residual limits (MRLs) for residues of drugs, pesticide and other chemical in the relevant tissues of food producing animals to protect and safeguard human health.

β-lactam antibiotics are widely used in veterinary medicine for treating bacterial infection in livestock farming. β- lactams consist mainly of two classes: penicillins and cephalosporins. Regarding our study about ceftiofur sodium as a member of cephalosporins, it is the third-generation cephalosporin with broad spectrum bactericidal activity against gram negative, gram positive and anaerobic pathogens [3]. However, ceftiofur sodium is widely used for treating and preventing diseases of most domestic animals. To our knowledge, there are little experimental data about its residues in rabbit tissues which was frequently detected [4].

Material and Methods

Animals and Experimental design

A total of twenty-four healthy male New Zealand White rabbits of 2±10 kg BW were used in this study. The animals were housed in batteries at Post Graduate Research Laboratory, Pharmacology Department, Faculty of Veterinary Medicine, Zagazig University and provided with a drug-free pelleted diet and *ad libitum* water. No clinical abnormalities were observed on rabbits during the experimental period. Rabbits were divided into two groups. The first group (n = 21) weighed and injected intramuscularly with Ceftiofur (Maxfur®, Kahira Pharm and Chem Ind Co, Egypt) at a dose of 2.2 mg/kg BW for five successive days [5], while the second group (n = 3) was kept as a control for preparation of blank and spiked samples.

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Three rabbits were slaughtered on the 1st, 3rd, 5th, 7th, 9th, 15th and 21stday after the last dose and samples were collected from blood, heart, lung, liver, spleen, thigh muscle, pectoral muscle and kidney for determining Ceftiofur residues.

Analytical procedures

Blood samples were centrifuged at 3500 r.p.m for 10 minutes to obtain clear serum, and then it was transferred immediately to sterile tubes and stored at -20^{0}C until analysis. Tissue samples were blended in a food processor for 20–30 seconds at high speed and were subjected to stirring to obtain a uniform paste-like consistency and then stored at -20^{o}C until analysis.

Extraction was carried out according to Chung *et al.* [6]. Frozen samples were thawed at room temperature (23°C). One milliliter of serum was mixed with 5 mL of 0.4% dithioerythritol (Sigma Aldrich Company) prepared in borate buffer and put in incubator at 50^{0}C for 15 minutes with good mixing. Five milliliters of 14% iodoacetamide (Sigma Aldrich Company) prepared in phosphate buffer was added and incubated for 30 minutes in the dark. One gram of tissues was mixed with 14 mL of 0.4% dithioerythritol in borate buffer pH 9 and incubated at 50^{0}C for 15 minutes with mixing. One and half milliliters of extract was mixed with 0.5 mL of 14% iodoacetamide in phosphate buffer and put in incubator for 30 minutes in the dark. Suspensions were acidified with phosphoric acid to pH 2.5. Centrifugation at 3500 r.p.m for 25 minutes at 4^{0}C was performed. Supernatant loaded to solid phase extraction cartridges after preconditioning with 1 mL methanol and 5 mL of phosphate buffer then the cartridges were washed with 5 mL of phosphate buffer and then 3 mL of each of 0.01 M NaOH and elution solution (15% acetonitrile) was added. The collection tubes were removed and 15 mL of water were added to give a total volume of 18 mL then loaded to SPE cartridges preconditioned with 2 mL methanol and 2 mL 25:75 methanol: 0.1 M NaCl and washed with 1 mL of water. Elution with 2.5 mL of 5:95 acetonitrile and 5% acetic acid in water was carried out. The collection tubes were removed from manifold and 10 mL of water were added to give a total volume of 12.5 mL then loaded to SPE cartridges preconditioned with 1 mL methanol and 2 mL 25:75 methanol: 0.1 M $CaCl_2$. Wash with 2 mL of water was done. The SPE cartridges were allowed to dry with high vacuum for up to 30 seconds. Elution with 2.5 mL 5:95 acetonitrile: 0.1 M NaCl was performed and 50 µL of supernatant was added to auto-sampler vial for analysis.

Liquid chromatographic conditions

The injection volume: 50 µL, flow rate: 1 mL/min., column temperature: 35^{o}C, UV-detector: 292 nm and the mobile phase: acetonitrile: De-ionized water: Trifluoroacetic acid (25:75:0.1%).

Quantification

Quantification of the residues was obtained and calculated from area under curves extrapolated automatically by the software.

Method validation

System Precision

It was conducted using 5 replicates of the toluene standard solution. Acceptance criteria: Relative standard deviation (RSD) ≤ 1% according to International conference on harmonization of technical requirements for registration of pharmaceuticals for human use (ICH, 2005) [7].

Linearity and range

Linearity was performed by preparing 8 different concentrations of drug standard. Linearity is defined by the squared correlation coefficient, which should be 0.99 (r^2) according to ICH.

Method Precision

It was conducted using 5 replicates of Ceftiofur standard solutions. Acceptance criteria: RSD ≤ 1% according to ICH.

Selectivity and specificity

Verification of selectivity was conducted by evaluating the spiked standard response following extraction from different rabbit tissues. Acceptance criteria: there is no interference between the pure standard and peaks of any impurities or extracted solvents according to (ICH).

Accuracy and recovery

The tissue samples of rabbits were spiked by added known quantities of ceftiofur. Those samples were analyzed against standard solutions of same concentrations. The accuracy was then calculated from the test results as a percentage recovery.

Limit of detection (LOD)

It was the concentration which gives signal to noise ratio 3:1 according to (ICH).

Limit of quantification (LOQ)

It was the concentration which gave signal to noise ratio 10:1 according to (ICH).

Ruggedness

It was conducted by the analysis of the same samples under different conditions, such as different personnel and different times. Acceptance criteria: pooled RSD is not more than 6% in every change item.

Robustness

It was determined by observing how a method stands up to slight variations in normal operating parameters. Acceptance criteria: pooled RSD is not more than 6% in every change item.

Results

Method validation

The HPLC system was found precise as the Relative Standard Deviation (RSD) of 5 replicates of the toluene standard solution was 0.002%. High correlation coefficient was obtained indicating linearity (r^2 = 0.99945). The method for ceftiofur separation was precise as the Relative Standard Deviation (RSD) of 5 replicates of ceftiofur standard solution was 0.23%. There was no interference between the pure standard and peaks of any impurities or extracted solvents. The retention time (R.T.) of ceftiofur was 4.975 minutes (Figure 1).

The percentage recovery of Ceftiofur spiked samples ranged from 95-102 %. The LOD was 0.0025µg/mL, while LOQ was 0.008µg/mL. The pooled RSD was 2.6 % for Ruggedness and the Pooled RSD for Robustness was 1.5 %.

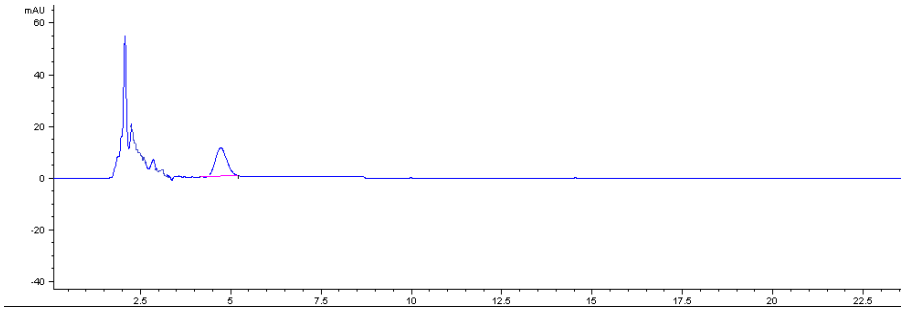

Figure 1: Chromatogram of Ceftiofur spiked muscle at a concentration of 2µg/gm automatically using HPLC.

Standard curve preparation

Ceftiofur standard concentrations of 0.025, 0.05, 0.1, 0.2, 0.5, 1, 2 and 5 µg/gm were prepared in homogenized muscles of control rabbits (blank samples) and then treated according to the described extraction procedure and their corresponding peak responses (Table 1 and Figure 2). The calibration curve calculated by linear regression equation method as y = 133.219612 X x- 4.865624 where y and x refered to the area under peak and the concentrations of ceftiofur, respectively. Linearity existed wihtin the range of 0.025 and 5 µg/gm with a correlation coefficient (r^2) of 0.99945.

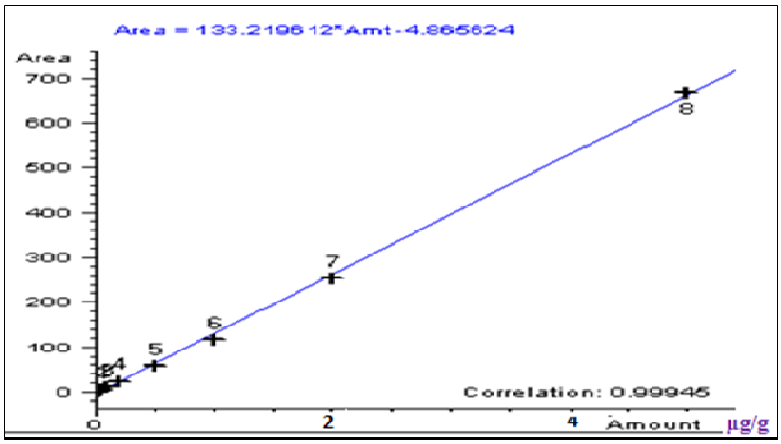

Figure 2: Standard curve of Ceftiofur automatically using HPLC.

Tissue residues

There was a wide distribution of the drug in the investigated tissues (liver, kidney, pectoral and thigh muscles, lung, spleen and heart) and serum. Ceftiofur concentrations were 3.17±0.17, 4.95±0.05, 1.68±0.06, 1.077±0.04, 2.187±0.08, 0.734±0.05, 1.9±0.18 µg/gm and 6.18±0.09 µg/mL on the 1st day post administration in liver, kidney, pectoral and thigh muscles, lung, spleen, heart and serum, respectively. Ceftiofur remained detectable till the 7thday in most examined tissues (kidney and lung) and serum while in kidney, it remained till the 9th day post treatment (Table 2).

Discussion

There is an increasing concern about the effects of drug residues mainly β-lactams (penicillins, cephalosporins and the newer β-lactam antibiotics) on human health which might produce immune-allergic reactions or development of resistance against certain pathogenic organisms [8-10]. Therefore, the European Community was established maximal residual limits (MRLs) for each drug to ensure consumers safety. In the present investigation, kidney, liver and lung contained the highest drug residue concentrations, while the lowest concentrations found in spleen, pectoral muscle and thigh muscle at the 1st day after the last dose administration. These results are slightly agreed with that reported by El-Sayed *et al.* [11] who mentioned that kidney and liver have the highest drug concentrations, while the lowest was in thigh muscle at the 1st day after stoppage of intramuscular administration of ceftiofur at a dose of 10 mg/kg BW for 5 days. Also, our results are in agreement with Chung et al., [6] who stated that kidney contained the highest drug residue concentration. Also, they reported that the drug residue concentration in muscle (0.071±0.01 µg/g) was more than in liver (0.066±0.01 µg/g) at the 1st day post-drug medication (4 mg/kg BW subcutaneously for 1 day) which was disagreed with our result.

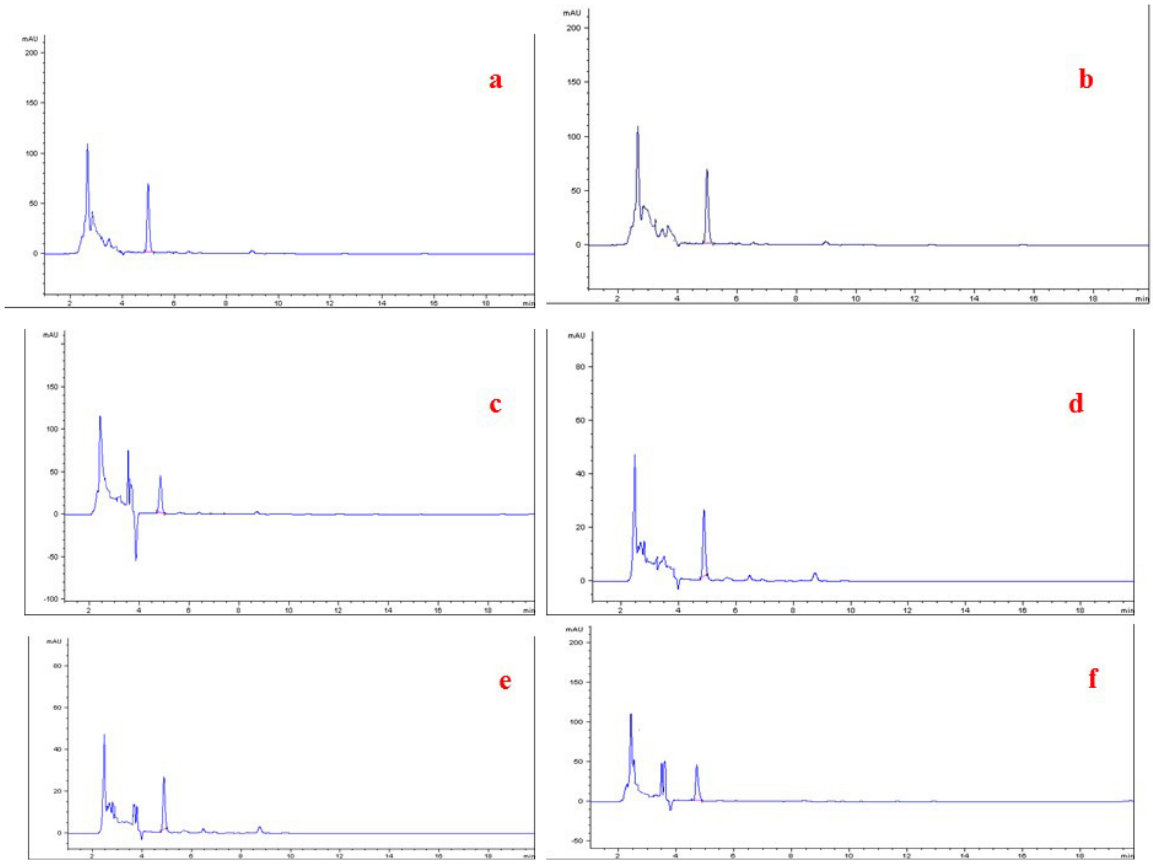

Figure 3: Chromatograms of ceftiofur extract of rabbit (a: kidney, b: liver, c: lung, d: pectoral muscle, e: thigh muscle and f: heart on 1st day post the last oral dose (2 mg/kg BW) using HPLC.

Table 1: The concentrations of Ceftiofur spiking in blank muscle (µg/gm) and their corresponding peak response automatically using HPLC.

RT*	Level	Amount (µg/gm)	Area
4.975	1	0.025	4.0690
	2	0.050	6.8300
	3	0.100	10.862
	4	0.200	24.444
	5	0.500	57.238
	6	1.000	115.27
	7	2.000	251.87
	8	5.000	667.95

*RT: Retention Time

Ceftiofur remained detectable till the 5th day in most examined tissues and continued to the 7th day in lungs and kidneys but still detected in kidneys till the 9th day post drug treatment. A progressive order of ceftiofur levels was detected in muscles, liver and kidney respectively with increasing time. The obtained results are supported by Peng *et al.* [12] who detected that the ceftiofur residue concentration in different tissues were in the following order: kidney > liver > lung > muscle after intramuscularly administration to 30 healthy pigs at a dose of 5 mg/kg for 3 successive days. Also, our results are supported by El-Sayed *et al.* [11] who indicated that the elimination rate of ceftiofur in skin > muscle > liver > kidney after

repeated intramuscular injections at a dose of 10 mg /kg BW every 24 hours for five consecutive days in normal and experimentally infected chickens with *Escherichia coli*. Our results agreed with Beconi-Barker *et al*. [13] who found that the highest concentrations were observed in the kidneys (10.68 and 6.33 µg/g for the 6.76 and 4.41 mg doses, respectively) followed by the injection sites, lungs, liver and muscle in twelve mixed-breed swine (26.5-42.5 kg) received ceftiofur hydrochloride at a dose of 3.08 mg/kg BW daily for 3 successive days and they noted that the lung is the clinical target organ, therefore ceftiofur is used for the treatment and control of swine respiratory diseases. Beconi-Barker *et al*. [14] reported that sheep cleared the drug more rapidly through the urine and the highest residue concentration was found in kidneys (9.016±1.153 µg/g, 0.29% of the administered dose) after 5 intramuscular doses at 2.2 mg of ceftiofur sodium/kg BW. Also, Tantituvanont *et al*. [15] found that the distribution of ceftiofur in pig tissues after intramuscular administration of ceftiofur hydrochloride at a dose of 3 mg/kg BW was predominantly in kidneys followed by the injection site, lung, liver, fat and muscle in pigs infected with porcine reproductive and respiratory syndrome virus (PRRSV) versus clinically healthy pigs. Thus, there is an increase in ceftiofur concentration in the lung, which considered the clinical target tissue in the treatment of swine respiratory diseases. Gilbertson *et al*. [16] mentioned that the highest residues were detected in kidneys with an average of 4.47±0.81 ppm and hence this tissue is considered the target tissue and the lowest residues were observed in muscles (the tissue most consumed by the public) with an average of 0.76±0.24 ppm.

Table 2: Ceftiofur concentrations in tissues of rabbits on various intervals post-treatment with 2.2 mg/kg BW once daily for 5 consecutive days automatically using HPLC. (Mean ± SE) (n=3)

Tissue	Ceftiofur concentration (µg/gm) Time-post treatment						
	1st	3rd	5th	7th	9th	15th	21th
Liver	3.17±0.17	1.26±0.16	0.46±0.03	ND	ND	ND	ND
Kidney	4.95±0.05	2.21±0.17	1.53±0.16	0.937±0.07	0.065±0.004	ND	ND
Pectoral muscle	1.68±0.06	0.968±0.06	0.237±0.02	ND	ND	ND	ND
Lung	2.187±0.08	1.045±0.05	0.212±0.04	0.059±0.004	ND	ND	ND
Thigh muscle	1.077±0.04	0.746±0.04	0.096±0.01	ND	ND	ND	ND
Spleen	0.734±0.05	0.136±0.02	ND	ND	ND	ND	ND
Serum	6.18±0.09	3.47±0.19	1.57±0.12	0.74±0.06	ND	ND	ND
Heart	1.9±0.18	0.924±0.07	0.06±0.01	ND	ND	ND	ND

ND= not detected

In our study, ceftiofur concentration in serum was higher than the corresponding concentrations in all other examined tissues. This finding disagreed with El-Sayed *et al*. [11] who stated that ceftiofur concentration in serum was lower than the corresponding concentrations in all other examined tissues in treated chickens. The difference may be due to different animal species, route of administration, dose, analytical techniques and pathological status.

Ceftiofur residues were lower than that MRL recommended by EMEA/CVMP [17] which is 1000, 2000 and 6000µg/kg in muscle, liver and kidney, respectively at the 3rd day post- injection. In the same line, El-Sayed *et al*. [11] supported the present finding as they mentioned that treated chickens must not be slaughtered before 3 days from the last dose of ceftiofur, while Beconi-Barker *et al*. [14] found that the total ceftiofur-related residues in sheep tissues 12 h after the end of five daily intramuscular injections at a dose of 2.2 mg/kg were lower than half the MRL defined by the Food and Drug Administration (1991) and is considered safe.

The acceptable daily intake (ADI) of ceftiofur = 20 µg/kg BW × 60 kg (standard body weight of human) = 1200 µg/person which can be consumed by human over a life time without appreciable risk. This was established by the Committee for Veterinary Medicinal Products (CVMP) based on the

MIC_{50} of 2.0 µg/mL for the most sensitive strains of human gut flora (*Escherichia coli, Lactobacillus* spp. and *Clostridium* spp.).

Conclusion

Ceftiofur residues were below the recommended MRL in pectoral muscle, thigh muscle and liver samples on the 3rd day post treatment while ceftiofur residues were below the recommended MRL on the 1st day post treatment in kidney. Therefore, muscles and livers of rabbits could be eaten safely on the 3rd day post-treatment while kidneys could be eaten safely on the 1st day post treatment without any health hazards on consumers as the residual level below the recommended MRL (6000 µg/kg in kidney, 1000 µg/kg in muscle and 2000 µg/kg in liver)

Conflict of interest

None of the authors have any conflict of interest to declare

Acknowledgment

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الملخص العربي

بقايا السفتيفيور صوديوم فى أنسجة الأرانب

السيد أحمد عبدالعزيز[1]، سامح محمد النبتيتى[1]، عبدالعظيم محمد عبدالسلام[2] و محمد أحمد ماهر[2*]
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استهدفت هذه الدراسة قياس بقايا السفتيفيور □وديوم فى الأنسجة المختلفة للأرانب وذلك بعد الحقن العضلى بالسفتيفيور □وديوم بجرعة2.2مجم/ كجم من وزن الأرانب. تم استخدام عدد أربع وعشرون أرنبا وتقسيمهم الى مجموعتين: المجموعة الأولى عبارة عن واحد وعشرون أرنبا تم حقنهم بالسيفتيفيور لمدة خمسة أيام متتالية- المجموعة الثانية عبارة عن ثلاثة أرانب وتم استخدامهم كمجموعة ضابطة. تم ذبح عدد 3 أرانب عند اليوم الأول، الثالث، الخامس، السابع، التاسع، الخامس عشر والحادى و العشرين بعد آخر جرعة ثم أخذ الأنسجة المختلفة (الكبد، الكلى، العضلات، الطحال، الرئة، القلب) والدم ثم فحصها وقياس مستوى الدواء فيها بواسطة جهاز الفصل الكروماتوجرافى السائل العالى الأداء. لقد وجد السيفتيفيور □وديوم فى الأنسجة المختلفة (الكبد، الكلى، العضلات، الطحال، الرئة، القلب) والدم حتى اليوم الخامس من إعطاء الجرعة النهائية للدواء بينما ظل مستمرا فى الكلي والرئة حتى اليوم السابع واستمر فى الكلى حتى اليوم التاسع. ومن هذه النتائج نستنتج أن الكبد والعضلات فى الأرانب المعالجة يمكن استهلاكها فى اليوم الثالث بينما الكلى يمكن استهلاكها فى اليوم الأول من بعد اعطاء الجرعة النهائية لأنها أقل من المعدلات المسموح بها.