



Research Article

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Residues of Ceftiofur in Healthy Rabbits after Intramuscular Administration

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ABSTRACT

The study was performed to quantitatively determine ceftiofur residues in different rabbit's tissues and to estimate the withdrawal time after intramuscular injection of the drug at a dose of 2.2 mg/kg BW by using high performance liquid chromatography technique. A total of twenty healthy male New Zealand White rabbits were used in this study, the rabbits were divided into two groups; the first group (n=15) was injected with ceftiofur for five successive days, while the second group (n = 5) remained untreated with any type of medication (control). Liver, kidney, muscle, heart, blood and lung samples from each rabbit were collected on the 1st, 3rd, 5th and 7th day post treatment. The results indicated a wide spread distribution of ceftiofur in the tested tissues, which remained within the detectable level till the 3rd day in the investigated tissues (heart, muscle and serum), while still detected till the 5th day post treatment in liver, kidney and lung respectively. Therefore, it is recommended that rabbits treated with ceftiofur must not be slaughtered before the end of the fifth day after the last dose administration in order to be safe for human consumption.

Key words: Ceftiofur, Residues, Rabbits, HPLC.

INTRODUCTION

Ceftiofur is a member of cephalosporins has important applications to both human and veterinary medicine, it is a third-generation cephalosporin with broad spectrum bactericidal activity against both gram negative, gram positive and anaerobic pathogens [1, 2].

As well as other cephalosporins, ceftiofur is bactericidal in vitro, its action result from inhibition of cell wall synthesis of susceptible multiplying bacteria [3].

However, the use of ceftiofur results in high concentrations of residues in different animal tissues which may cause allergy in hypersensitive people. Low doses of antibiotics in feed stuffs can lead to problems such as spread of drug-resistant micro-organisms if consumed for long periods [4].

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.

Besides environmental pollution [5], high concentrations of drug residues in edible animal tissues resulted from the extra-label use of drug or non-compliance with the withdrawal period [6].

The control of drug residues is a significant point to obtain safe food for human consumption, therefore, maximum residue limits (MRL) of drugs have been set for edible animal tissues [7, 8].

The rabbit is one of the most beneficial animals in the world at converting food for meat [9]. Rabbit meat is valuable for its nutritional properties because it is lean, rich in protein of high biological value, low in cholesterol content. So rabbit meat is healthier than others meat frequently used in human nutrition [7].

Therefore, the aim of present work was to determine residues of ceftiofur in different rabbit tissues and serum following multiple intramuscular administration of this drug using High Performance Liquid Chromatography (HPLC). Moreover, the estimation of the withdrawal time of the drug in rabbit tissues was determined.

MATERIALS AND METHODS

Experimental design

A total of 20 apparently healthy male New Zealand White rabbits (2-2.5 kg B.WT) were used in this study. The animals were housed in batteries and provided with a drug-free pelleted diet and ad libitum water for at least 15 days before the study to ensure complete excretion of any drugs from their bodies. No clinical abnormalities were observed on rabbits during the experimental period. They were divided into 2 groups, The first group of (n= 15) rabbits were injected intramuscularly with ceftiofur at a dose of 2.2 mg/kg once daily for 5 consecutive days [10] while the second group (n=5) rabbits served as controls (they were used for preparation of blank sample and spiked sample).

Materials

Ceftiofur sodium (Kenafur 1gm) was obtained as sterile powder. It is manufactured by Kahira Pharm. HPLC grade methanol (FISHER SCIENTIFIC, United Kingdom), HPLC grade acetonitrile (ARLO ERBA reagent, Spain) and deionized water used for HPLC analysis was purified through a Milli-Q system (Waters Corp., Milford, MA).

Tissue samples:

Three rabbit were slaughtered on the 1st day, 3rd day, 5th day and 7th day after the last dose of drug administration and samples were collected from blood, heart, lung, liver, muscle and kidney for determining Ceftiofur residues.

Analytical procedures

Calibration curve:

The standard curve of ceftiofur in rabbits' serum and deionized water was linear between 0.155 and 0.31 µg/ml, 0.62, 1.25 µg/ml, 2.5 µg/ml, 5 µg/ml, 10 µg/ml and 20 µg/ml respectively.

Assay of serum samples

Extraction of ceftiofur from rabbit serum was carried out according to [11]. This method for the quantification of the total concentration of ceftiofur involved a deproteinization of the serum and a back – extraction of acetonitrile with dichloromethane. 400 µl of acetonitrile was added to 200 µl of serum for deproteinization and vortex – mixed. After centrifugation of the samples for 10 minutes at 10000g, the supernatant was brought into a new Eppendorf vial. Then 600 µl of dichloromethane was added. After vortex – mixing for 15 seconds, the sample were again centrifuged at 10000 g, for 10 minutes. The top layer was transferred into an auto sampler vial.

Assay of tissue samples

The extraction of ceftiofur from rabbit tissues was carried out according to Junza et al. [12] which is a modified method from Granelli & Branzell [13], Chico, et al. [14]. Accurately weighed 4 grams of finely minced rabbit tissues (used after thawing) placed into 50 ml polypropylene test tube. Then 10 ml acetonitrile: water (80:20, v/v) was added and the mixture was centrifuged 3000 rpm for 5 minutes. The suspended solution was decanted into 50 ml glass tube and nitrogen drying, then reconstituted with 2 ml of water. The mixture was filtered through 0.45 µm nylon membrane filter. The filtrate was put into auto-sampler vial and analyzed under the same chromatographic condition as plasma samples.

Condition of High Performance Liquid Chromatography (HPLC)

According to Elham et al. [15] Ceftiofur in both collected blood and tissue samples were assayed using high performance liquid chromatography. Mobile phase: The injection volume of 20 µL, flow rate of 1.0 mL/min., column temperature of 35°C, UV- detector of 292 nm and the mobile phase: acetonitrile: De-ionized water: Trifluoroacetic acid (25:75:0.1%). Quantification of residues in the samples was performed and calculated from area under the curve, and extrapolated automatically by the software

RESULTS

Standard curve concentration:

Ceftiofur calibration curve was prepared at concentrations of 0.155 and 0.31 µg/ml, 0.62, 1.25 µg/ml, 2.5 µg/ml, 5 µg/ml, 10 µg/ml and 20 µg/ml respectively. Linearity existed within the range of 0.155 µg/ml and 20 µg/ml with

a correlation coefficient (r^2) of 0.99997. The retention time (R.T.) of ceftiofur was 10.45 minutes (Figure 3). The percentage recovery of ceftiofur spiked samples ranged from 90-94%. The LOD for ceftiofur was 0.00403 $\mu\text{g/ml}$, while, LOQ was 0.012 $\mu\text{g/ml}$.

Table 1. Area under the curve corresponding to pure standard ceftiofur concentration ($\mu\text{g/ml}$).

Retention time	Level	Area	Concentration($\mu\text{g/ml}$)
10.45min	1	7.700	0.155
	2	15.438	0.31
	3	30.600	0.62
	4	61.437	1.25
	5	122.680	2.5
	6	245.990	5.0
	7	499.540	10
	8	1002.800	20

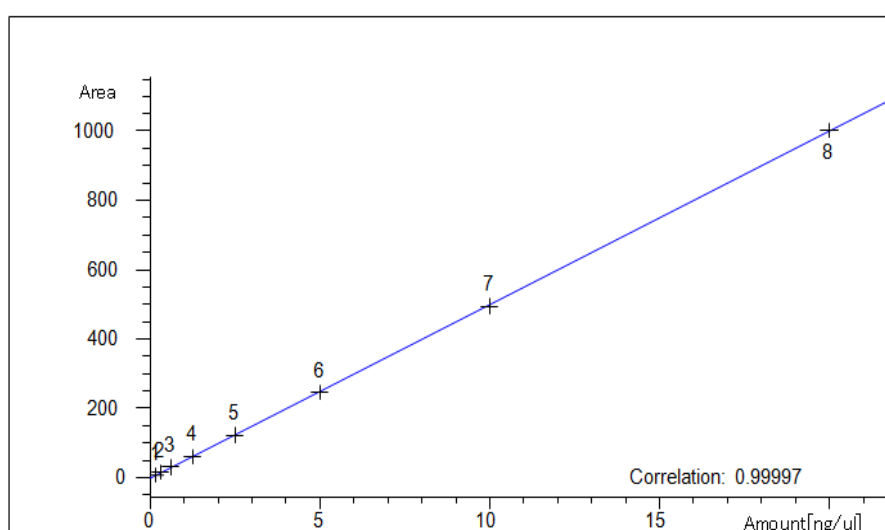


Figure 1: standard curve of ceftiofur determined automatically using HPLC chromatogram system

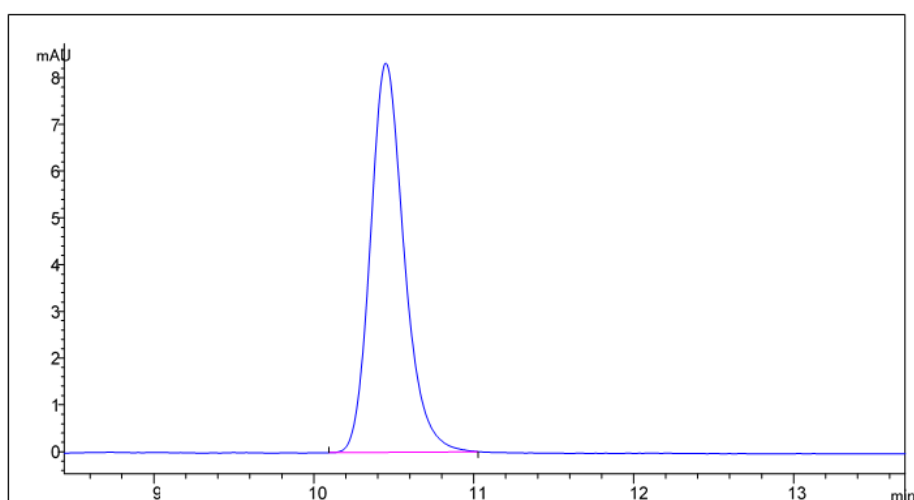


Figure 2: Chromatogram of pure standard Ceftiofur (2.5 $\mu\text{g/ml}$) in rabbit serum determined automatically using HPLC chromatogram system

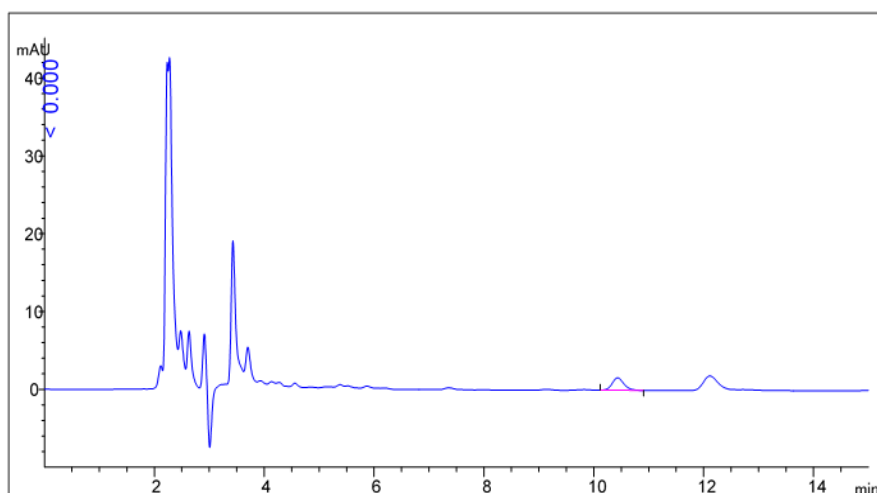


Figure 3: Chromatogram of ceftiofur residue in rabbit serum sample after intramuscular administration at a dose of 2.2mg/kg b. wt once daily for 5 consecutive days.

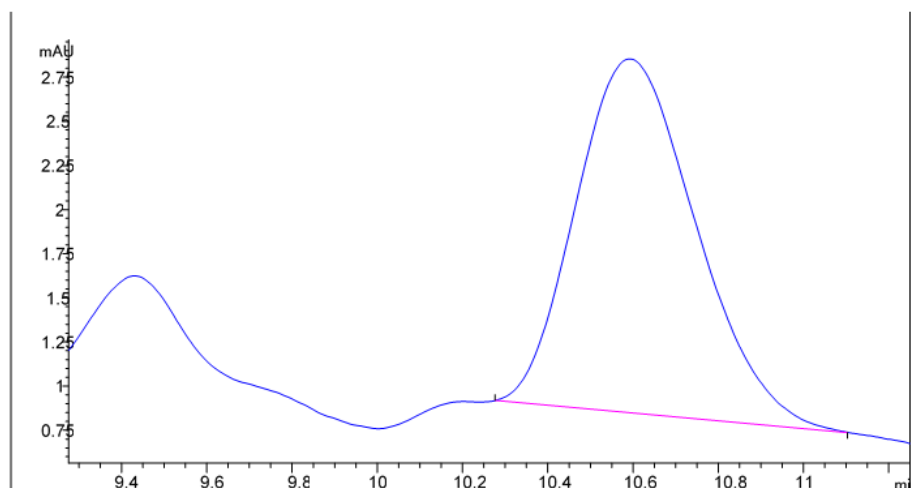


Figure 4: Chromatogram of ceftiofur residue in rabbit muscle sample after intramuscular administration at a dose of 2.2mg/kg b.wt once daily for 5 consecutive days.

Results of tissue residues:

Tissue samples from liver, kidney, lung, heart, muscle, and serum were taken for assaying of residues of ceftiofur on 1st day, 3rd day, 5th day and 7th day after the last intramuscular administration of 2.2 mg/kg.b.wt from normal rabbits. The data showed that there was a wide distribution of the drug in the investigated tissues. Ceftiofur concentrations were $(0.74 \pm 0.08, 4.87 \pm 0.57, 0.31 \pm 0.04, 0.63 \pm 0.03, 0.44 \pm 0.06$ and 0.1 ± 0.01 $\mu\text{g/gm}$) on the 1st day post administration in liver, kidney, muscles, lung, heart and serum, respectively. Ceftiofur remained detectable till the 5th day in most examined tissues (kidney, liver and lung) while in muscle, serum and heart it remained till the 3rd day post treatment (Table 2).

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

Tissues \ Day	1st day	3rd day	5th day	7th day
Serum ($\mu\text{g/gm}$)	0.1 ± 0.01	0.02 ± 0.04	N.D	N.D
Muscle($\mu\text{g/gm}$)	0.31 ± 0.04	0.04 ± 0.05	N.D	
Liver($\mu\text{g/gm}$)	0.74 ± 0.08	0.16 ± 0.02	0.05 ± 0.01	N.D
Kidney($\mu\text{g/gm}$)	4.87 ± 0.57	1.16 ± 0.22	0.15 ± 0.09	N.D
Lung($\mu\text{g/gm}$)	0.63 ± 0.03	0.22 ± 0.06	0.07 ± 0.05	N.D
Heart($\mu\text{g/gm}$)	0.44 ± 0.06	0.15 ± 0.01	N.D	N.D

N.D: non detectable

DISCUSSION

There is an increasing importance about the effects of drug residues mainly β -lactams antibiotics as 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 [16-18].

Therefore, the European Community was established maximal residual limits (MRLs) for each drug to avoid human health hazard and to ensure that consumers safety.

The current study revealed that ceftiofur was highly distributed in various body tissues. In the present investigation kidney ($4.87 \pm 0.57 \mu\text{g/g}$), liver ($0.74 \pm 0.08 \mu\text{g/g}$), and lung ($0.63 \pm 0.03 \mu\text{g/g}$) contained the highest drug residue concentrations, while the lowest concentrations found in muscle ($0.31 \pm 0.08 \mu\text{g/g}$), serum ($0.1 \pm 0.01 \mu\text{g/g}$) and heart ($0.44 \pm 0.06 \mu\text{g/g}$) at the 1st day after the last dose administration. These results slightly agreed with that recorded by Li et al. [19] and El- sayed, et al. [20] who found the highest concentration in kidney ($2.589 \mu\text{g/g}$) at 12 hours, their results indicated that the elimination rate was skin/ fat > muscle > liver > kidney > injection site, with the elimination half-life of 28.99, 35.80, 36.76, 55.72, 160.8 hours, respectively.

Also, our results are partially agreed with Chung et al. [21] who found that kidney contained the highest ceftiofur concentration. Also, they reported that the drug residue concentration in muscle ($0.071 \pm 0.01 \mu\text{g/g}$) was more than in liver ($0.066 \pm 0.01 \mu\text{g/g}$) at the 1st day after stopping drug medication in duck (4mg/kg BW subcutaneously for 1 day) and this may be attributed to differences in dosage or species.

The results of this study were similar to Beconi–barker, et al. [22] who observed that the highest concentration of ceftiofur found in kidney followed by injection sites, liver, lung and muscle in twelve mixed – breed swine (26.5-42.5 kg) which received ceftiofur hydrochloride at dose of 0.38mg/kg b.wt daily for 3 successive days . Also [23] compatible with this study found the distribution of ceftiofur in pig tissues where found kidney is predominant tissue has residue of ceftiofur followed by injection site, liver, lung, fat and muscle after administration of ceftiofur hydrochloride at dose of 3 mg /kg b.wt in pigs infected with respiratory syndrome virus versus and porcine reproductive clinically healthy pigs .

The obtained result are supported with Beconi-Barker, et al. [24] who detected that after 5 intramuscular doses at 2.2mg of ceftiofur sodium /kg b.wt in sheep the drug more rapidly through the urine and kidney with highest residue concentration ($9.016 \pm 1.135 \mu\text{g/g}$).

Ceftiofur remained within detectable limit till 3rd day in most tested tissues and continued to 5th day in kidney, liver and lung after the last dose of drug administration; this result is supported by Peng et al. [25] who found ceftiofur concentration in different tissues in the following order: kidney, liver, lung then muscle after intramuscular administration of 5 mg /kg for 3 consecutive day to 30 healthy pigs.

Also, our results are supported by Riediker and Stadler [18] 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*.

Fortunately, our results came in consistency with those obtained by Gilbertson et al. [26] who mentioned that the highest residues were detected in kidneys with an average of $4.47 \pm 0.81 \text{ ppm}$ 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 $0.76 \pm 0.24 \text{ ppm}$ after intramuscular administration of ceftiofur sodium at dosage of 3-5mg/kgb.wt for 3 days in swine.

With the same findings, San–Martin, et al. [27] illustrated that the absorption and elimination of cefquinome in serum and tissue of salmon after single doses of 5, 10 and 20 mg/kg were administered intra-peritoneally to 30 fish. the maximum concentration occurred in the following order; kidney followed by liver, serum, muscle then brain.

In our study, ceftiofur concentration in serum was lower than the corresponding concentrations in all other examined tissues. This finding agreed with El- sayed et al. [20] who stated that ceftiofur concentration in serum was lower than the corresponding concentrations in all other examined tissues in treated chickens. And this result supported by Yein et al. [28] who found that residue of ceftiofur in the plasma peaked 2 h after the last dose at 23.12 ppm and had fallen to 10.65 ppm by 12 h. after intramuscular injections of ceftiofur sodium at 5 mg/kg body weight to twelve pigs were given 3 times at 24 h intervals and killed 12 h after the last dose.

In our study, it has been shown that kidney and liver contained the highest drug concentrations of ceftiofur ($4.87, 0.74 \mu\text{g/g}$ respectively), while the lowest drug concentrations were found in muscle and serum ($0.31, 0.1 \mu\text{g/g}$ respectively), 24 hours after stopping drug medication. This result slightly agreed with [29] who found that

the highest tissue concentration of cefquinome were present in kidney and liver. Cefquinome was not detected in most tissues of broilers at the 5th day following the last dose but was detected in kidney, liver and lung. Cefquinome completely disappeared from all tissues at 7th day after stopping drug medication. These finding suggested that cefquinome is excreted mainly by kidney [27, 30].

On the same direction, Abd El-Aty et al. [31] studied tissue residue of ceftazidime in rabbit after intramuscular injection of 50mg/kg b.wt twice daily for five consecutive days and they found that the tissue level concentration of the drug were highest in kidney followed by liver, heart the muscle and plasma and this supported the present finding.

The obtained result are supported by Niehaiying et al. [32] who found that bioavailability after administration of ceftiofur sodium to 5 healthy adult buffalo at dose (2.2 mg/kg). Concentrations of ceftiofur in plasma were determined by using of high-performance liquid chromatography. There was good absorption and rapid elimination of ceftiofur from plasma.

Interestingly, Chung et al. [33] found that residues of ceftiofur sodium in the blood and edible tissues (plasma, muscle, liver, and fat tissues) of healthy ducks at a dose rate of 2 mg/kg body weight ceftiofur 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 ($0.059 \pm 0.01 \mu\text{g/g}$).

On the other hand, the present finding disagree with Meegan et al. [34] who reported that ceftiofur residue persisted in plasma for 5 days using High performance liquid chromatography (HPLC) with tandem MS and this may be due to using the dose rate of 6.6 mg/kg would be maintain drug level $0.61\mu\text{g/ml}$ in plasma for 5 days.

Ceftiofur residues were lower than that MRL recommended by Codex Alimentarius Commission (CAC/ MRL) [35] (1000, 2000 and 6000 $\mu\text{g/kg}$ in muscle, liver and kidney, respectively) at the 1st day after the treatment; this findings were supported by Beconi- barker et al. [24] who 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 and is considered safe while El-Sayed et al.[20] mentioned that treated chickens must not be slaughtered before 3rd days from the last dose of ceftiofur.

Also, San-Martin, et al. [27] reported that the withdrawal time was 104 h after single intraperitoneal administration of cefquinome at dosage of 20mg/kg in Coho Salmon fish. Moreover, Abd -El-Aty et al. [31] found that no ceftazidime residues were detected in tissues and plasma after 72 h from intramuscular injection of 50mg/kg b.wt, twice daily for 5 consecutive days in rabbits.

CONCLUSION

According to the established MRL of ceftiofur, the obtained results revealed that ceftiofur concentrations in the examined organs were lower than the recommended MRL at the 1st day post treatment. Rabbits treated with ceftiofur must not be slaughtered before the fifth day from last dose of repeated administration of ceftiofur to withdraw the drug residues from all tissues of treated rabbits to be safe for human consumption.

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