Detection of Tilmicosin Residues by HPLC and its Effect on Cardiac Enzymes and Hematology in Broiler Chickens

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Abstract

The aim of the present study was to detect the tilmicosin residues in muscles, kidneys and liver of broiler chickens and to investigate its impact on both cardiac enzymes and some hematological parameters. Seventy broiler chickens were divided into two groups; the first was left as a negative control. The second group was given tilmicosin orally (30 mg/kg BW) once/day for three successive days. Reversed phase-high performance liquid chromatography (RP-HPLC) with UV detector at 287 nm and solid phase extraction were used for detecting drug residues in tissue samples. Results indicated a widespread distribution of tilmicosin in most tested tissues. All tissue samples were considered tilmicosin free at the 9th day after the last oral dose except liver. Tilmicosin elicited a significant reduction in red blood cells count, hemoglobin concentration, packed cell volume, lymphocytes and eosinophiles. On the other hand, heterophiles count and the mean corpuscular volume were increased significantly. White blood cells, monocytes, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration showed non-significant changes. Levels of all cardiac enzymes (Aspartate aminotransferase, lactate dehydrogenase, creatine kinase-MB and Troponin I) were high.

Keywords: Tilmicosin Residues, Hematological Parameters, Cardiac Enzymes, Broiler, HPLC

Introduction

Tilmicosin is a broad-spectrum bacteriostatic macrolide antibiotic synthesized from tylosin for veterinary use only. It is predominantly effective against Mycoplasma spp., Pasteurella spp. and various Gram-positive organisms [1,2]. Tilmicosin is advised to be the drug of choice in respiratory infections specially in M. gallisepticum, Ornithobacterium rhinotracheale and P. multocida in broiler chickens [3,4].

Tilmicosin caused cardiovascular toxicity and deaths by an intravenous administration or at doses much greater than therapeutic dose [5-7]. Its mode of action is through inhibition of protein synthesis by binding to the 50 S ribosomal subunit of sensitive microorganisms [8]. According to the European Medicines Agency and the veterinary drug residue regulations of the Chinese Ministry of Agriculture, the maximum residue levels (MRLs) of tilmicosin in broiler chicken muscles, kidneys and liver were 0.075, 0.25 and 1.0 µg/g, respectively [9,10]. Abu-Basha et al. [4] mentioned that intravenous injection of tilmicosin (15 mg/kg BW) in chickens caused a very serious cardiovascular effects (cardiac toxicity and necrosis of the cardiac muscle) and death.

Consumers eat tissues (milk, meat and eggs) which have antibiotic residues for a long time that can produce bacterial resistance and therapeutic failures among them. Similarly, administration of very low doses of some drugs for a prolonged time produce reproductive and teratogenic effects [11]. Not only the macrolides residues in edible products have direct toxic effects but also may lead to allergic reactions and development of resistant bacteria in humans [12].

The aim of this study was to investigate the residues of tilmicosin in broilers' tissues and its...
side effects on cardiac enzymes and hematological parameters.

**Material and Methods**

**Experimental animals and design**

Seventy healthy Hubbard broiler chickens, 4 weeks of age and 1150-1200 g weight were used. Chickens were obtained from a private poultry farm in Cairo. They were divided equally into two groups and housed in batteries (160 cm length, 140 cm width and 50 cm depth) at the graduate research laboratory, Animal Health Research Institute, Dokki. The first group was kept as a negative control and used for preparation of blank and spiked samples for validating the method. The second group was given tilmicosin phosphate (250 mg/mL, Advotil AC, CHEMVET, Advanced Agrochemicals and Veterinary Products Industrial Co., Amman, Jordan) directly into the crop in a dose of 30 mg/kg BW once/day for three consecutive days. Five chickens were sacrificed at 1st, 3rd, 5th, 7th, 9th and 14th day following the last oral dose. Samples from liver, muscles and kidneys were taken for quantitative determination of tilmicosin residues. Two blood samples were collected; the first 2 mL were collected with EDTA from the wing vein at 1st, 3rd, 5th and 7th day following the last oral dose for hematological examination. While, the second one (3 mL) was obtained at 1st, 3rd and 5th day following the last oral dose to estimate the cardiac enzymes in the serum (Creatine kinase-MB, Lactate dehydrogenase, Aspartate aminotransferase and Troponin I).

**Analytical procedures**

**Preparation of samples for analysis**

At the time of assay, frozen chicken tissue samples were partially thawed at room temperature (23°C) for 30 min and were minced and homogenized in the mincer for 1 min.

**Drug residues extraction**

Extraction of the drug residues from the samples was carried out according to Zhang et al. [13]. Ten mL of acetonitrile were added to 5 gm of the homogenized sample in a centrifuge tube (50 mL), then shaking for 20 min and centrifugation for 10 min at 3500 rpm were carried out. The supernatant was transferred into a 50 mL polypolyethylene centrifuge tube. Five milliliters of monobasic potassium phosphate buffer and 8 mL of acetonitrile were added to the tissue pellet and thorough shaking of the mixture for 20 min was carried out. Centrifugation for 10 min at 3500 rpm was then performed. Supernatants were combined with 40 mL of HPLC water. The mixture solution was centrifuged at 3500 rpm for 10 min. The supernatant was introduced to solid phase extraction (SPE) cleanup step. The SPE was conditioned with 10 mL of methanol then 10 mL of deionized water and the sample was applied to the cartridge. The flow rate was not more than 2 drops/s. The cartridge was not allowed to dry at this step, so the cartridge was flushed with 10 mL water then 10 mL of acetonitrile was applied. The SPE cartridge was dried for at least 3 min under vacuum. Elution was performed successively with 2.5 mL Ammonium acetate (0.1 mol/L)/methanol/ acetonitrile solution. The eluated solution was evaporated until dryness by a nitrogen stream at 30°C in a water bath. The sample was reconstituted by 1 mL dipotassium hydrogen phosphate buffer, mixed and filtered through 0.45 µm filters before injection into HPLC.

Liquid chromatography operating conditions was adjusted for 100 μL injected volume, flow rate, 0.7 mL/min; wave length, 287 nm; column temperature, ambient; stop time: 30 min; post time: 6 min. The mobile phase A was 0.05% trifluoroacetic acid while mobile phase B was acetonitrile (gradient conditions) as at 0 minute 71% from mobile phase A and 29% from B and at 11 min; 54.5% from A and 45.5% from B. Finally; 50% was taken from mobile phase A and the same from B at 11.5 and 14 minutes.

**Quantification**

Quantification of residues in the samples was obtained and calculated from the area under curves extrapolated automatically by the software (ChemStation, Germany).
Validation method

It is the evaluation process used to ensure that the performance characteristics of an analytical procedure are to demonstrate that it is suitable for its intended purpose.

System Precision: It was conducted using five replicates of the caffeine standard solution with acceptance criteria of Relative Standard Deviation (RSD) ≤ 1% according to the International Conference on Harmonization of technical requirements for registration of pharmaceuticals for human use (ICH).

Linearity and range: Linearity was performed by preparing a minimum of five different concentrations of drug standard and defined by the squared correlation coefficient, which should be 0.99 ($r^2$) according to ICH.

Precision method: It was conducted using five replicates of tilmicosin standard solutions with acceptance criteria of RSD ≤ 1% according to ICH.

Selectivity and specificity: Verification of selectivity was conducted by evaluating the spiked standard response following extraction from different chicken tissues. Regarding the 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 chickens were spiked by adding known quantities of tilmicosin. Those samples were analyzed against standard solutions of the corresponding concentrations. The method was accurate according to the calculated test results from the % recovery.

Limit of detection (LOD): It is the concentration which gives signal to noise ratio 3:1 according to ICH.

Limit of quantification (LOQ): It is the concentration which gives 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, different times, etc. Acceptance criteria: pooled RSD is not more than 6% in every change item.

Robustness: It was determined by observing how an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. Acceptance criteria: pooled RSD is not more than 6% in every change item.

Results

Method validation results

The HPLC system was found precise because the RSD of five replicates of caffeine standard solution was 0.001%. High correlation coefficient was obtained indicating linearity ($r^2= 0.99606$). The method for separating tilmicosin was precise as the RSD of seven replicates of tilmicosin standard solution was 0.498%. There was no interference between the pure standard and peaks of any impurities or extracted solvents. The retention time (R.T.) of tilmicosin was 6.86 min (Figure 1A). The percentage recovery of tilmicosin spiked samples ranged from 97-99%. The LOD for tilmicosin was 0.015 µg/mL, while, LOQ was 0.045 µg/mL. The pooled RSD for tilmicosin was 4%.

Standard curve preparation

Tilmicosin standard concentrations of 0.05, 0.1, 0.2, 0.5, 1, 2 and 5 µg/mL and their corresponding peak responses were illustrated in Table (1) and Figure (1B). The calibration curve was calculated by linear regression equation method as $y= 590.419507x – 10.3641$ where y symbol indicated the area under peak and x symbol indicated concentrations of tilmicosin. Linearity existed within the range of 0.05 and 5 µg/mL with a correlation coefficient $r^2=0.99606$. 
Table 1: The concentrations of Tilmicosin standard (µg/mL) and their corresponding peak response

<table>
<thead>
<tr>
<th>RT*</th>
<th>Level</th>
<th>Amount (µg/mL)</th>
<th>Area under curve</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.86</td>
<td>1</td>
<td>0.050</td>
<td>39.3</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.100</td>
<td>78.6</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.200</td>
<td>134.2</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>0.500</td>
<td>231.2</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>1.000</td>
<td>680.1</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>2.000</td>
<td>975.8</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>5.000</td>
<td>3003.1</td>
</tr>
</tbody>
</table>

*RT: Retention time

**Tissue residues**

Tilmicosin distribution in tissues was represented in Table (2) and Figure (1 C-E). The represented data emphasized a widespread distribution of the drug in tested tissues (liver, kidneys and muscles). The highest concentration of tilmicosin residues was detected in the liver (3.84 ± 0.35 µg/gm) followed by kidneys (2.28 ± 0.13 µg/gm) while the lowest concentration was detected in the muscles (1.33 ± 0.12 µg/gm) at the first day after the last oral dosage. Tilmicosin remained within the detectable level till the 7th day in most tested tissues but disappeared in muscles, while in liver; it remained till the 9th day after treatment with the drug.

**Hematological and biochemical results**

Tilmicosin elicited significant decrease in most blood parameters (RBCs, Hb, PCV, lymphocytes, eosinophiles), while heterophiles and MCV showed significant increase. However, no significant change was recorded in WBCs, MCH, MCHC and monocytes. Values of all the tested cardiac enzymes (AST, LDH, CK-MB and Troponin I) were highly elevated (Tables 3 and 4).

Table 2: The concentrations of tilmicosin in tissues of sacrificed broilers at various intervals after treatment (30 mg/kg BW once daily for 3 consecutive days) (n=5)

<table>
<thead>
<tr>
<th>Tissues</th>
<th>The concentration (µg/gm) mean ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1st</td>
</tr>
<tr>
<td>Liver</td>
<td>3.84 ± 0.35</td>
</tr>
<tr>
<td>Kidneys</td>
<td>2.28 ± 0.13</td>
</tr>
<tr>
<td>Muscles</td>
<td>1.33 ± 0.12</td>
</tr>
</tbody>
</table>

*ND=Not detected
Discussion

Tilmicosin has been used for treatment of respiratory diseases in cattle, swine [14], rabbits [15] and rats [16]. The effect of tilmicosin (30 mg/kg BW once daily for 3 successive days) on the tissue residues following its oral administration was recorded. Following the oral administration, tilmicosin was detected in all tested tissues of normal healthy chickens following the last oral dose. Macrolides are generally hepatotoxic [17]. These results are consistent with Zhang et al. [13] who mentioned that the liver is the most concerned organ for tilmicosin residues in broiler chickens. Also, Fricke et al. [18] reported that the highest level of tilmicosin was found in liver followed by kidneys, lungs and muscles.

The maximum residue levels (MRLs) of tilmicosin in broiler chicken muscles, kidneys and liver are 0.075, 0.25, and 1.0 μg/g, respectively [9,10]. The reported withdrawal time is 10 days. In this study, tilmicosin could not be detected at the 9th day after the last dose except in liver with a concentration of 0.28±0.05 μg/g which is approximately the quarter of the mentioned level. Our results were comparable with Stobba-Wiley et al. [19] who modified a validated method for quantitation of tilmicosin residues in swine, cattle, and sheep edible tissues, also chicken fat, skin, and muscles over a concentration range of 0.025–20 μg/g. For chicken kidneys and liver, the method was developed over a range of 0.060–20 μg/g, compared with our results; the lower concentrations ranged from 0.07-0.28 μg/g in muscles, kidneys and liver. According to Zhang et al. [13], a minimum withdrawal time of tilmicosin was 9 days which indicated that the residue levels in muscle, liver and kidney tissues were below MRL. This was partially in agreement with the results obtained during the current study.

In the present study, tilmicosin caused decrease in red blood cells (RBCs), hemoglobin (Hb) and packed cell volume (PCV). In accordance, Yazar et al. [20] reported that tilmicosin caused significant decrease in RBCs counts in rabbits. However, our results disagreed with Xie et al. [21] and Yazar et al. [22] who mentioned that tilmicosin did not influence RBCs, hematocrit (HCT) value, mean corpuscular volume (MCV) and Hb. Also, our results were incomparable with Elsayed et al. [23] who reported that Hb and PCV concentration did not change with the administration of tilmicosin. In addition, the obtained results showed no significant change on MCH. Similarly, Xie et al. [21] found that tilmicosin had no significant change on the mean corpuscular hemoglobin (MCH).
Table 3: Effect of tilmicosin (30 mg/kg BW once daily for 3 consecutive days) on hematological parameters of broiler chicken (mean±SE)

<table>
<thead>
<tr>
<th>T</th>
<th>G</th>
<th>Hb  (g%)</th>
<th>RBCS x10⁶/µl</th>
<th>PCV</th>
<th>MCV (fl)</th>
<th>MCH (Pg)</th>
<th>MCHC %</th>
<th>WBCs</th>
<th>Eosinophile</th>
<th>Heterophiles</th>
<th>Lymphocyte</th>
<th>Monocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st day</td>
<td>G1</td>
<td>12.9±0.22</td>
<td>3.1±0.03</td>
<td>34.46±0.3</td>
<td>111.22±1.12</td>
<td>41.62±0.65</td>
<td>37.42±0.42</td>
<td>8.4±0.18</td>
<td>0.69±0.01</td>
<td>24.9±0.11</td>
<td>72.18±0.05</td>
<td>2.23±0.07</td>
</tr>
<tr>
<td></td>
<td>G2</td>
<td>12.26±0.05</td>
<td>3.36±0.02</td>
<td>34.18±0.04</td>
<td>101.75±0.62</td>
<td>36.5±0.32</td>
<td>35.4±0.03</td>
<td>7.84±0.08</td>
<td>0.66±0.01</td>
<td>29.48±0.18</td>
<td>67.62±0.18</td>
<td>2.24±0.07</td>
</tr>
<tr>
<td>3rd day</td>
<td>G1</td>
<td>13.14±0.04</td>
<td>2.96±0.05</td>
<td>34.04±0.25</td>
<td>115.15±1.22</td>
<td>44.7±0.62</td>
<td>38.6±0.28</td>
<td>8.8±0.24</td>
<td>0.65±0.01</td>
<td>25.1±0.2</td>
<td>71.9±0.21</td>
<td>2.35±0.04</td>
</tr>
<tr>
<td></td>
<td>G2</td>
<td>11.64±0.04</td>
<td>2.54±0.02</td>
<td>32.18±0.23</td>
<td>126.7±0.47</td>
<td>45.8±0.47</td>
<td>36.9±0.39</td>
<td>8.48±0.15</td>
<td>0.53±0.01</td>
<td>29.32±0.35</td>
<td>67.86±0.3</td>
<td>2.29±0.07</td>
</tr>
<tr>
<td>5th day</td>
<td>G1</td>
<td>13.04±0.07</td>
<td>3.1±0.02</td>
<td>34.08±0.19</td>
<td>109.96±0.72</td>
<td>42.08±0.39</td>
<td>38.27±0.29</td>
<td>8.8±0.13</td>
<td>0.67±0.01</td>
<td>25.32±0.34</td>
<td>71.7±0.28</td>
<td>2.31±0.04</td>
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<td></td>
<td>G2</td>
<td>10.48±0.05</td>
<td>2.32±0.03</td>
<td>27.82±0.24</td>
<td>119.95±0.62</td>
<td>45.22±0.57</td>
<td>37.69±0.32</td>
<td>8.1±0.14</td>
<td>0.56±0.02</td>
<td>31.5±0.51</td>
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<td>2.4±0.04</td>
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<tr>
<td>7th day</td>
<td>G1</td>
<td>13.04±0.09</td>
<td>3.12±0.03</td>
<td>34.22±0.26</td>
<td>109.74±0.95</td>
<td>41.8±0.26</td>
<td>38.12±0.27</td>
<td>8.6±0.23</td>
<td>0.67±0.01</td>
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<td>71.42±0.11</td>
<td>2.29±0.02</td>
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<tr>
<td></td>
<td>G2</td>
<td>9.36±0.07</td>
<td>2.08±0.03</td>
<td>24.68±0.26</td>
<td>118.68±0.33</td>
<td>45.05±0.58</td>
<td>37.95±0.42</td>
<td>7.5±0.15</td>
<td>0.54±0.01</td>
<td>31.3±0.44</td>
<td>65.76±0.46</td>
<td>2.4±0.06</td>
</tr>
</tbody>
</table>

T: time, G: group; G1: control; G2: tilmicosin; Hb: Hemoglobin; RBCs: red blood cells; PCV: packed cell volume; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; WBCs: white blood cells; WBCs, Eosinophile, Heterophiles, Lymphocyte and Monocytes were presented x10⁶/µl; *significant P<0.01 and **significant P<0.001.

Table 4: Effect of tilmicosin on cardiac enzymes of broiler chickens (Mean±SE)

<table>
<thead>
<tr>
<th>Time</th>
<th>Group</th>
<th>LDH (U/L)</th>
<th>CK-MB (U/L)</th>
<th>AST (U/L)</th>
<th>Troponin I (µg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st day</td>
<td>G1</td>
<td>67.4±0.66</td>
<td>129±0.5</td>
<td>15.52±0.11</td>
<td>5.8±0.26</td>
</tr>
<tr>
<td></td>
<td>G2</td>
<td>87.2±0.61**</td>
<td>147.4±0.66**</td>
<td>24.84±0.15**</td>
<td>79.2±0.61**</td>
</tr>
<tr>
<td>3rd day</td>
<td>G1</td>
<td>67.8±0.52</td>
<td>127.2±0.57</td>
<td>15.42±0.08</td>
<td>8.2±0.41</td>
</tr>
<tr>
<td></td>
<td>G2</td>
<td>90.4±0.66**</td>
<td>205.12±0.5**</td>
<td>27±0.12**</td>
<td>87.6±0.53**</td>
</tr>
<tr>
<td>5th day</td>
<td>G1</td>
<td>63±0.67</td>
<td>128.4±0.36</td>
<td>15.36±0.09</td>
<td>9±0.39</td>
</tr>
<tr>
<td></td>
<td>G2</td>
<td>99.4±0.36**</td>
<td>227.4±0.6**</td>
<td>28.42±0.12**</td>
<td>92.8±0.61**</td>
</tr>
</tbody>
</table>

G: group; G1: control; G2: tilmicosin; LDH: Lactate dehydrogenase enzyme; CK-MB: Creatine Kinase MB; AST: Aspartate Aminotransferase enzyme; *significant P<0.01 and **significant P<0.001.
In the current study, there was no significant change in white blood cells (WBCs) count and monocytes on the 1st, 3rd, 5th and 7th days. The effect of tilmicosin on leukocytes is controversial. Xie et al. [21] confirmed that tilmicosin did not show any significant change in WBCs and monocytes. However, Yazar et al. [20] reported that tilmicosin caused statistically significant decrease in WBCs of rabbits and chicken. Also, our findings revealed a highly significant decrease in lymphocytes and eosinophiles count. On the other hand, heterophiles count showed a highly significant increase. The previous results were incompatible with Xie et al. [21] who reported that tilmicosin did not change neutrophils, lymphocytes, eosinophiles and basophiles.

Measurement of the cardiac enzymes (AST, LDH and CK-MB) has been used since long time to detect cardiac and skeletal muscle injury, but these three biomarkers lack sensitivity and specificity [24, 25]. Although increased CK-MB activity may be responsible for the myocyte damage, its elevation alone does not necessarily indicate the myocyte damage [26]. Therefore, troponin I was measured and in general, increased CK-MB activity was associated with increased troponin I level [27]. It is accepted that troponin I may be a more sensitive marker than CK-MB in myocyte damage [28-30]. In this study, tilmicosin illustrated marked increase in all cardiac enzymes (LDH, CK-MB, AST and troponin I) on 1st, 3rd and 5th days post treatment. These results were supported by Yazar et al. [23] who mentioned that tilmicosin caused a significant increase in cardiac creatine kinase activity. Also, Ibrahim and Abdel-Daim [31] reported that tilmicosin intoxication increased serum cardiac injury biomarkers LDH, CK and CK-MB. Moreover, Elazab et al. [32] stated that biochemical results demonstrated marked increase in serum aspartate transaminase (AST), lactate dehydrogenase (LDH), creatinekinase (CK) activities and cardiac troponin T (cTnT) concentrations in tilmicosin-treated rats indicating severe cardiotoxicity. However, our results were dissimilar to those previously reported by Jordan [33] who recorded non-significant changes in serum creatinine level, AST and ALT in cattle treated with tilmicosin. Also, with Altunok et al. [34] who reported that tilmicosin has no negative effects of on biochemical variables of rabbits. The difference may be due to species and dose difference.
Conclusion

The present study clearly demonstrated that liver is the concerned tissue for tilmicosin residues in broiler till the 9th day post treatment. Tilmicosin elicited significant decrease in most of blood parameters. In addition, tilmicosin induced elevation in cardiac markers.

Conflict of interest

None of the authors have any conflict of interest to declare.

Acknowledgment

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References


الملخص العربي
تحديد البقايا الدوائية للتميكيوزين بواسطة جهاز التحليل الكروماتوجرافي العالي الكفاءة وتآثره على انزيمات القلب ومكونات الدم في دجاج التسمين

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قسم الفارمكولوجيا - كلية الطب البيطري - جامعة الزقازيق - مصر

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استهدفت هذه الدراسة قياس بقايا التتميكيوزين في أنواع نمط التسمين في كبد الدجاج، الكلى والعضلات بعد إعطاء عدد 70 دجاجة تميكيوزين 25 مجم 3 مرات في اليوم لمدة 3 أيام متتالية. تم ترتيب الدجاجان المجموعة الأولى ونظام التجربة تم التعرف على مدة سحب الأدوية من الأنسجة المختلفة تتعلق بالعظام الكبيرة للأسلاك الأدمي. تم تجربة 6 دجاجات واخذ عينات من الدم عند اليوم الأول، الثاني، الثالث، الخامس، السابع والرابع خلال 10 أيام. تم استخراج عينة واحدة من الأنسجة المختلفة (الكبد، الكلى، الغدد) في كل من الجهاز الفصل الكروماتوجرافي السائل العامل بوصفة دراسة. وقد أفادت النتائج إزالة التتميكيوزين في أنواع نمط التسمين المختلفة السابق ذكرها. كما تم ملاحظة وجود التتميكيوزين حتى اليوم الخامس في بعض الأنسجة، كما تم إعطاء جرعة عالية من الأنسجة المختلفة (الكبد، الكلى، الغدد) في كل第二天ة من إعطاء جرعة تسمى بالكميات المسمى بالكلقايرة. باستخدام هذه النتائج، يمكن للأطباء استنفار التتميكيوزين قبل إعطاء الأدوية للمريض. هذه الدراسة تظهر إمكانية استخدام التتميكيوزين كفرع للعلاج في حالات محددة.