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STUDIES ON OXYTETRACYCLINE RESIDUES DEPLETION IN RABBIT MEAT

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ABSTRACT

A total of 100 rabbits weighed about 1000-1500 grams (free from any antibiotics by feeding the rabbits on a balanced ration free from antibiotics for three weeks) were divided into two groups ; control group (50) and test group (50) which were injected subcutaneously with oxytetracycline hydrochloride 20mg/kg body weight for five successive days. 5 rabbits from each group were slaughtered at zero time, 24hr, 48hr, 72hr, 96hr, 120hr, 144hr, 168hr (1week), 2weeks and 3weeks. Residue depletion of oxytetracycline was determined using high performance liquid chromatography (HPLC). The slaughter time strongly affects the concentration of oxytetracycline residues in animal meats, so that by increasing the slaughter time the oxytetracycline residues decreased. Boiling for 30 minutes caused a great degradation of oxytetracyline residues in rabbit meats but not complete destruction. Freezing at -20° c caused a lower degradation than that caused by boiling. So neither boiling nor freezing could be used as reliable methods to get rid of oxytetracycline residues in rabbit meats. The recovery rates for oxytetracycline in rabbit muscles, kidneys and livers were 102%, 92% and 86%, respectively, at a concentration of 20 µg/kg of spiked samples.

***Key words:** Oxytetracycline, Residues, Rabbit meat.*

INTRODUCTION

Rabbit meat is considered a high quality product due to its high protein and low saturated fatty acids, cholesterol and sodium content. Antibiotics are widely used in veterinary medicine and subsequently drug residues may persist in foods derived from animals, which may pose an adverse health effect for the consumer. Today antimicrobial drugs are used to control, prevent and treat infection, and to enhance animal growth and feed efficiency. Tetracyclines are most widely used antibiotics in veterinary medicine in Egypt due to its broad spectrum of antimicrobial activity, availability and low cost. Violation of the maximum residue limit (MRL) regulation can only be proven with fully validated chromatographic methods such as high performance liquid chromatography (HPLC) (Moats, 1997). The use of antimicrobials in food-producing animals may result in the presence of residues in foodstuffs of animal origin. Protection of public health against possible harmful effects of veterinary drug residues is a relatively recent preoccupation. The initial intention for adequate consumer protection led to the desire to achieve a complete elimination of all traces of drug residues in food commodities. Therefore, animal drugs were initially approved based on a "no residue" tolerance policy, but actually the "zero" tolerance represented the sensitivity of the analytical method used to monitor for drug residues. As analytical methods improved, the "no residue" tolerance was continually being lowered. Ultimately, a policy of negligible tolerance, based on toxicology data, was developed (Boisseau, 1993; Teske, 1993). The legislation for pharmaceutical and veterinary products established a withdrawal period of antibiotics of 28 days for fattening rabbits, limiting the addition of antibiotics to the first days of fattening (Badiola *et al.*, 2007). Since the most of foods-producing animals are always cooked before consumption and the variations in oxytetracycline levels in the meat are dependent on type of cooking. More findings about the effect of cooking on oxytetracycline residue are needed to accurately determine consumer exposure to this drug. The boiling for 30 minutes and roasting at 150°C for 30 minutes caused a complete degradation of drug residues. Freezing at -20°C ensured gradual degradation of the residues remained in different meats of the medicated rabbits (Gehad, 2002). The acceptable MRLs for tetracyclines as recommended by the Joint FAO/WHO Expert Committee on Food Additives is 200, 600, and 1200 µg/kg for muscles, liver, and kidney, respectively, (Mehran *et al.*, 2009). The abundant and improper use of tetracycline antibiotics may result in the presence of their residues in edible animal meats, which can be toxic and dangerous for human health and potentially cause allergic reactions. In addition, low-level doses of antibiotic in foodstuffs consumed for long periods can lead to the spread of drug-resistant microorganisms (Yu *et al.*, 2011). Therefore, this work was designed to determine the residue depletion of oxytetracycline

in rabbit meats and the effect of boiling and freezing on oxytetracycline residues.

MATERIALS and METHODS

I. Materials:

1- HPLC grade acetonitrile, methanol and water.

2- AR/GR grade disodium hydrogen phosphate (Na2HPO4), citric acid and oxalic acid.

3- Oasis HLB cartridge 6 cm3 (200 mg).

4- From pure standard of oxytetracyline (assay 96.2% in HPLC), as their hydrochloride, individual stock standard solution at 1mg/mL (free base) was prepared in methanol in an amber color volumetric flask and was stored at -20°C in the dark for maximum period of 2 months. A composite working standard solutions of 300, 250, 200, 160, 80, 40 and 20 µg/mL were prepared by diluting stock solution with methanol. As it is unstable at room temperature, so prepared daily and stored at 4°C. For analysis, 0.01 M oxalic acid (pH 1.6), 0.1 M citric acid and 0.2 M disodium hydrogen phosphate (Na2HPO4) buffer were prepared in Milli-Q water and filtered through 0.22 µm cellulose filter.

5- 0.01 M methanolic oxalic acid (pH 1.86) was prepared in methanol.

6- McIlvaine buffer (pH 3.85) was prepared by mixing 278 mL of 0.1 M citric acid solution in 222 mL of 0.2 M Na2HPO4 solution and the pH was adjusted to 3.85 with extra citric acid solution. All these buffer solutions were stored at 4°C.

II. Methods:

1. Injection of rabbits with oxytetracyclines:

Hundred rabbits weighed about 1000-1500 grams(free from any antibiotics by feeding the rabbits on a balanced ration free from antibiotics for three weeks) were divided into two groups; control group (50) and test group (50) which were injected subcutaneously with oxytetracycline hydrochloride 20 mg/kg body weight for five successive days.

2. Slaughtering of rabbits:

Five rabbits from each group were slaughtered at zero time, 24 hr, 48 hr, 72 hr, 96 hr, 120 hr, 144 hr, 168 hr (1 week), 2 weeks and 3 weeks.

3. Effect of boiling and freezing on oxytetracycline residues in rabbit meats.

The samples were taken from meat, liver and kidney directly after slaughtering and were divided as follow:

1st group: Consists of 100 samples from each type (meat, liver and kidney).The oxytetracycline residues were determined using HPLC without treatment (boiling or freezing).

2nd group: Consists of 100 samples from each type (meat, liver and kidney).The oxytetracycline residues were determined using HPLC after boiling for 30 min.

3rd group: Consists of 100 samples from each type (meat, liver and kidney).The oxytetracycline residues were determined using HPLC after freezing at - 20° C for one, two and three months.

4. Determination of oxytetracycline residues by HPLC method:-

The method recommended by Biswas *et al.* (2007) was used.

4.1. Instrumentation:

HPLC and uv detector.The analytical column was a Luna 5 µ C8 (RP- C8) column (4.6 × 250 mm, 5µm particle size). The optimized mobile phase for desorption and separation was a mixture of 0.01 M oxalic acid/acetonitrile/methanol (77:18:5, v/v/v), and the flow rate was 0.6 mL/min. The detection was performed at 355 nm with scanning range 340-360 nm.

4.2. Method of extraction:

1- Frozen meat samples were thawed and finely diced with scissors after trimming off external fat and fascia.

2- The finely cut samples were blended in a high speed (15,000 rpm) meat blender for 2 min.

3- A representative portion of this sample (10 g) was weighed into a polypropylene tube and homogenized with 10 mL of Milli-Qwater for 1.5 min using Ultra-Turrex T25 meats homogenizer (Janke and Kenkel, IKA, LaborTecnik, USA).

4- Then an aliquot (0.5 g) of homogenized sample was transferred into a glass test tube, fortified with 50 µL of variable concentrations of the working standard solution, leaving the analytes in contact with meat sample for 30min.

5- After 3 mL of McIlvaine buffer was added, the mixture was vortexed at high speed, incubated for 5 minutes at room temperature and centrifuged at 3,500 rpm for10 min in a refrigerated centrifuge.

6- The extraction was repeated by adding 2 mL of McIlvaine buffer and the supernatant was pooled.

7- The supernatant was filtered and loaded on an Oasis HLB6 cm3 (200 mg) polymeric cartridge previously conditioned with 3 mL of methanol and 2 mL of water. The cartridge containing the sample was washed with 5 mLof water, and then tetracyclines were eluted with 4.5 mL of 0.01 M methanolic oxalic acid (pH 1.80).

8- One milliliter of eluent was filtered through 0.22 µm nylon filter, vortexed and centrifuged, and then 20 µL of the aliquot was injected into the HPLC system.

RESULTS

Table 1: Minimum, maximum and mean values of oxytetracycline hydrochloride residues in injected rabbits meat (µg/kg) before and after boiling (n=5).

Statistical analysis	Before boiling			After boiling		
	Min.	Max.	Mean ±S.D	Min.	Max.	Mean ±S.D
Zero time	2482.9	6235.1	4565.1±1.640	1865.3	4356.8	2842.1±0.988
After 1 day	3398.3	4213.5	3801.4±0.341	2227.4	4201.2	3126.9±0.885
After 2 days	2175.8	4325.4	3433.2±0.904	1958.5	4215.3	3219.4±0.984
After 3 days	2002.5	4124.5	3238.5±0.906	1869.5	4004.8	2845.5±1.013
After 4 days	1854.2	3612.4	2938.2±0.755	1625.1	3335.3	2474.7±0.665
After 5 days	2002.1	3400.8	2612.1±0.611	925.4	3014.6	1568.3±0.900
After 6 days	1432.1	2854.1	2024.5±0.559	652.9	2425.4	1321.1±0.747
After 1 week	896.9	2296.5	1533.6±0.636	432.6	2072.3	1071.9±0.733
After 2 weeks	N.D	421.6	198.2±0.204	N.D	93.3	45.3±0.042
After 3 weeks	N.D	6.8	1.4±0.003	N.D	N.D	N.D

Table 2: Minimum, maximum and mean values of oxytetracycline hydrochoride residues in injected rabbits meat (µg/kg) before and after freezing (n=5).

Statistical analysis	Before freezing			After freezing 1 month			After freezing 2 month			After freezing 3 month		
	Min.	Max.	Mean ±S.D	Min.	Max.	Mean ±S.D	Min.	Max.	Mean ±S.D	Min.	Max.	Mean ±S.D
Zero time	2482.9	6235.1	4565.1±1.640	2222.4	4021.3	3370.6±0.709	1741.1	2182.4	1950.7±0.166	421.6	1245.8	828.6±0.322
1 day	3398.3	4213.5	3801.4±0.341	2387.5	4012.8	3290.3±0.769	1532.6	2007.2	1771.1±0.198	386.1	965.7	645.4±0.223
2days	2175.8	4325.4	3433.2±0.904	2014.5	4214.9	3125.6±0.946	1333.7	1893.5	1606.1±0.220	210.3	855.9	539.5±0.244
3days	2002.5	4124.5	3238.5±0.906	1924.5	4100.3	2919.3±1.016	1144.7	1636.6	1396.2±0.174	184.2	533.6	346.9±0.147
4days	1854.2	3612.4	2938.2±0.755	1754.2	3421.8	2573.5±0.651	1055.1	1355.5	1192.9±0.114	102.1	333.6	195.2±0.089
5days	2002.1	3400.8	2612.1±0.611	1125.3	2928.4	1889.9±0.667	924.1	1163.3	1043.8±0.094	93.4	126.5	107.4±0.012
6days	1432.1	2854.1	2024.5±0.559	1175.2	2632.1	1705.9±0.664	785.1	932.2	871.3±0.064	66.2	114.6	87.3±0.019
1week	896.9	2296.5	1533.6±0.636	514.9	2154.7	1276.4±0.322	501.4	881.1	725.3±0.150	N.D	112	55.4±0.052
2weeks	N.D	421.6	198.2±0.204	N.D	134.8	60.8±0.059	N.D	8.7	1.7±0.003	N.D	N.D	N.D
3weeks	N.D	6.8	1.4±0.003	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D

Table 3: Minimum, maximum and mean values of oxytetracycline hydrochloride residues in injected rabbits kidneys (µg/kg) before and after boiling (n=5).

Statistical analysis	Before boiling			After boiling		
	Min.	Max.	Mean ±S.D	Min.	Max.	Mean ±S.D
Zero time	21356.4	42356.2	35355.5±8.524	9684.2	27321.4	14626.7±7.537
After 1 day	17852.4	28254.1	22811.7±4.275	7128.6	16589.2	10245.1±4.137
After 2 days	12182.3	20398.2	15329.5±3.619	4827.3	11324.1	7217.3±2.944
After 3 days	7928.3	18925.1	10929.2±4.680	5006.3	13842.1	7979.9±3.484
After 4 days	4625.1	12653.2	7409.4±3.579	3018.6	9897.2	5294.6±3.059
After 5 days	3483.7	9653.4	5872.9±2.677	2016	6591.6	3819.6±1.866
After 6 days	3521.3	8321.4	5247±2.011	1834.6	5914.6	3201±1.712
After 1 week	1632.5	3872.5	2540±0.894	593.4	2095.1	1155.5±0.620
After 2 weeks	264.8	1653.4	965.1±0.543	14.7	923.7	510±0.364
After 3 weeks	N.D	32.5	17.7±0.014	N.D	N.D	N.D

Table 4: Minimum, maximum and mean values of oxytetracycline hydrochloride residues in injected rabbits kidneys (µg/kg) before and after freezing (n=5).

Statistical analysis	Before freezing			After freezing 1 month			After freezing 2 month			After freezing 3 month		
	Min.	Max.	Mean ±S.D	Min.	Max.	Mean ±S.D	Min.	Max.	Mean ±S.D	Min.	Max.	Mean ±S.D
Zero time	21356.4	42356.2	35355.5±8.524	14215.6	36215.4	26964.3±8.531	4623.3	6229.3	5297.8±0.614	1339.4	2365.1	1834.3±0.416
1 day	17852.4	28254.1	22811.7±4.275	12549.7	21321.5	17324.9±3.796	3499.9	5277.1	4196.7±0.675	1047.2	1967.7	1482.6±0.386
2days	12182.3	20398.2	15329.5±3.619	9231.7	14328.1	10865.6±2.095	2901.3	3331.8	3077.2±0.162	1036.4	1756.3	1271.6±0.294
3days	7928.3	18925.1	10929.2±4.680	7010.7	14012.8	9108.7±2.812	2110.1	3185.7	2806.7±0.414	823.4	1389.1	1074.5±0.216
4days	4625.1	12653.2	7409.4±3.579	3392.1	11926.1	6242.7±3.962	1441.8	2314.4	1882±0.318	585.7	1330.1	911.7±0.308
5days	3483.7	9653.4	5872.9±2.677	2518.8	7325.2	4428.4±2.147	1263.6	2132.1	1696.6±0.377	503.3	963.1	767.8±0.199
6days	3521.3	8321.4	5247±2.011	2368.2	6821.4	4088.4±1.894	1085.8	1788.9	1384.5±0.308	239.4	634.4	466.3±0.152
1week	1632.5	3872.5	2540±0.894	1186.3	3102.8	1927.6±0.769	322.7	1232.6	701.7±0.434	62.4	322.7	187.1±0.118
2weeks	264.8	1653.4	965.1±0.543	28.4	1359.1	720±0.516	N.D	52.1	20.3±0.021	N.D	11.7	5±0.005
3weeks	N.D	32.5	17.7±0.014	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D

Table 5: Minimum, maximum and mean values of oxytetracycline hydrochloride residues in injected rabbits livers (µg/kg) before and after boiling (n=5).

Statistical analysis	Before boiling			After boiling		
	Min.	Max.	Mean ±S.D	Min.	Max.	Mean ±S.D
Zero time	5985.4	10238.1	7393.1±1.832	3751.4	7392.7	5196.9±1.781
After 1 day	4724.4	8963.2	6475.6±1.766	3009.7	6492.8	4418.1±1.449
After 2 days	3734.8	6362.8	4838.5±1.216	2821.7	4893.4	3454.9±0.834
After 3 days	3093.4	5932.4	4111.2±1.147	2494.7	4003.4	3223.4±0.598
After 4 days	2892.1	4682.9	3626.5±0.755	2005.4	3834.7	2821.9±0.781
After 5 days	2632.6	4321.4	3321.3±0.671	1870.8	3591.7	2615.6±0.662
After 6 days	2074.5	3745.2	2962.2±0.642	1558.4	2931.2	2164.4±0.525
After 1 week	1636.3	3112.5	2609.8±0.624	800.7	2451.3	1538.4±0.713
After 2 weeks	472.6	1421.8	1037.6±0.422	63.4	733	481.7±0.267
After 3 weeks	N.D	14.2	3.8±0.006	N.D	N.D	N.D

Table 6: Minimum, maximum and mean values of oxytetracycline hydrochloride residues in injected rabbits livers (µg/kg) before and after freezing (n=5).

Statistical analysis	Before freezing			After freezing 1 month			After freezing 2 month			After freezing 3 month		
	Min.	Max.	Mean ±S.D	Min.	Max.	Mean ±S.D	Min.	Max.	Mean ±S.D	Min.	Max.	Mean ±S.D
Zero time	5985.4	10238.1	7393.1±1.832	4782.9	9543.8	6545.5±2.021	3869.6	7213.5	5512.9±1.507	2614.6	4823.4	3328.5±0.875
1 day	4724.4	8963.2	6475.6±1.766	4392.5	7654.8	5949.1±1.327	3596.1	6366.6	4978.9±1.161	2300.7	4621.4	3112.7±0.909
2days	3734.8	6362.8	4838.5±1.216	3293.8	5721.8	4196.4±1.052	3187.3	5236.8	4353.6±0.849	2401.7	4321.5	2959.9±0.799
3days	3093.4	5932.4	4111.2±1.147	2814.9	5020.7	3637.3±0.874	2711.7	4917.4	3532.2±0.881	2187.9	3621.4	2581.5±0.588
4days	2892.1	4682.9	3626.5±0.755	2563.8	4324.9	3356.7±0.314	2222.1	4236.3	3096.9±0.822	1766.2	2824.5	2122.8±0.420
5days	2632.6	4321.4	3321.3±0.671	2111.9	3982.4	2918.6±0.715	2086.7	3775.2	2725.9±0.711	1448.1	2633	1908.7±0.451
6days	2074.5	3745.2	2962.2±0.642	1845.2	3222.3	2408.3±0.565	1735.1	3030.2	2261.6±0.518	1085.1	1845.5	1441.4±0.303
1week	1636.3	3112.5	2609.8±0.624	1184.7	2884.9	2157.7±0.655	1096.3	2785.4	1942.6±0.648	239.5	1366.9	719.1±0.474
2weeks	472.6	1421.8	1037.6±0.422	294.5	1293.1	899.8±0.437	N.D	1120.1	433±0.559	N.D	793.3	262.9±0.372
3weeks	N.D	14.2	3.8±0.006	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D

Min. = Minimum, Max. = Maximum, N.D = Not detected and S.D= Standard Deviation.

Figure1: HPLC chromatogram of meat sample of the group slaughtered at zero time after the last dose of oxytetracycline (6235.1 µg/kg).

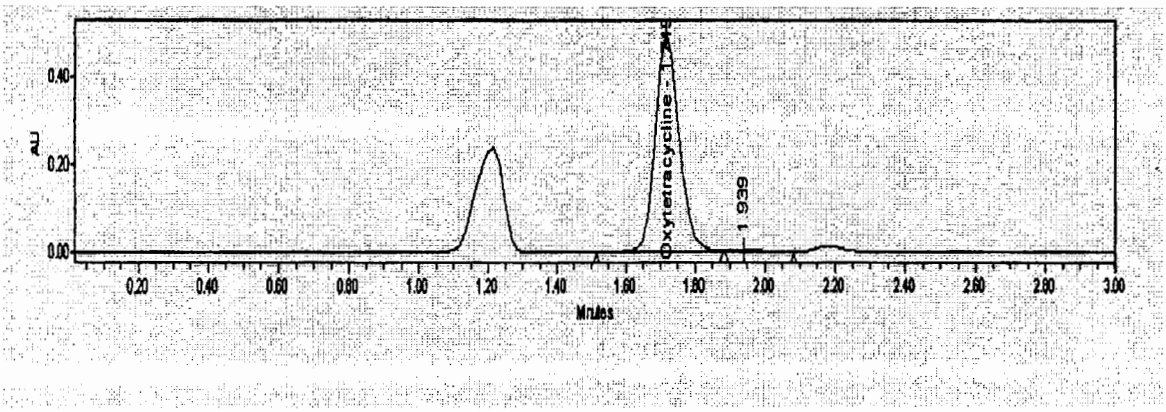


Figure 2: HPLC chromatogram of meat sample of the group slaughtered one weeks after the Last dose of oxytetracycline and freezed for three months (87.5 µg/kg).

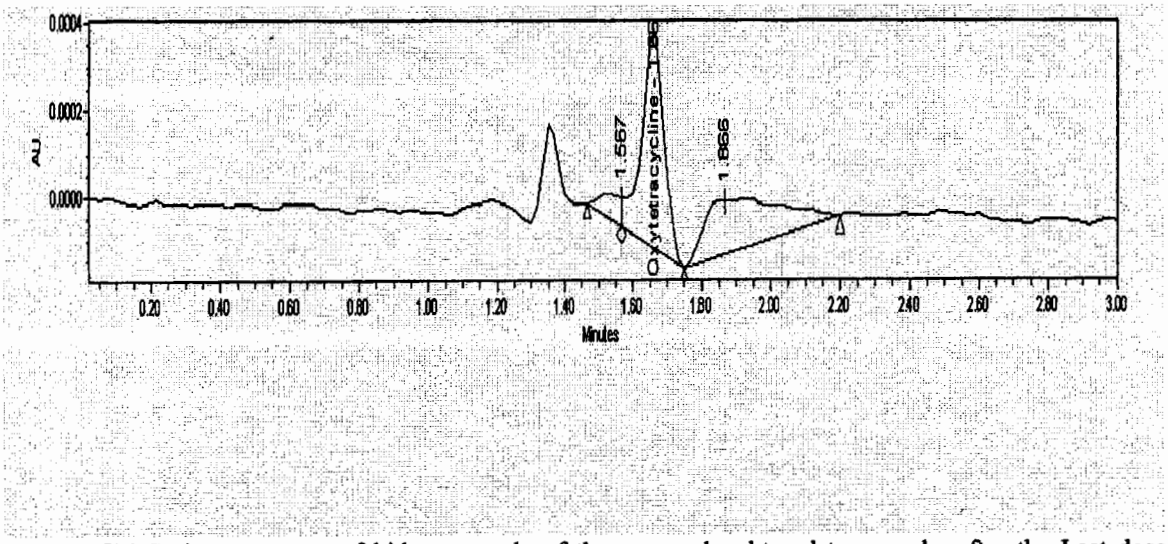
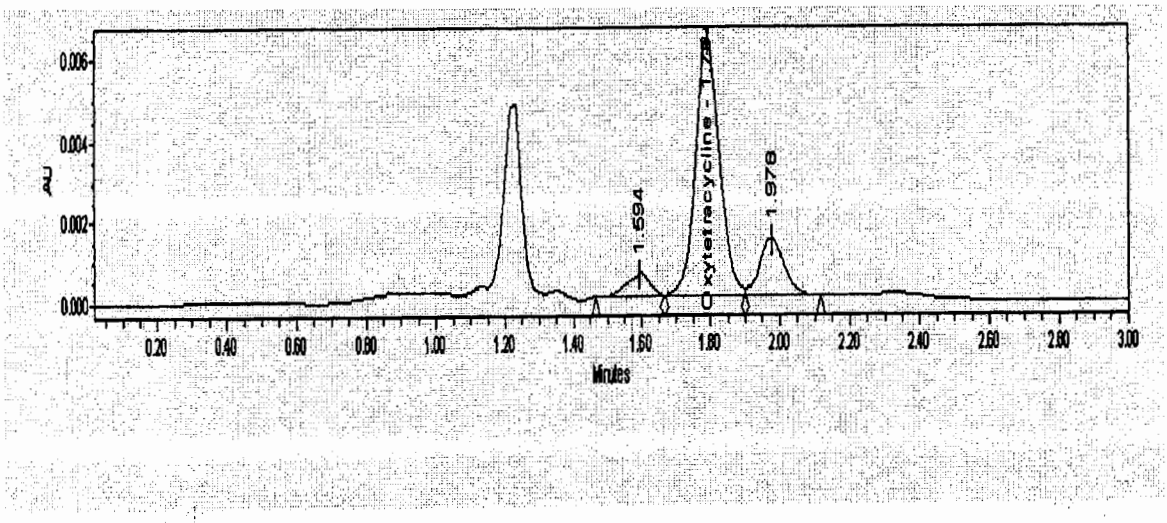


Figure 3: HPLC chromatogram of kidney sample of the group slaughtered two weeks after the Last dose of oxytetracycline and freezed for one month (438.2 µg/kg).



DISCUSSION

The obtained results in table (1) showed that the mean values of oxytetracycline in meat after slaughter time zero, one day, two days, three days, four days, five days, six days, one week, two weeks and three weeks from the last dose (20 mg/kg body weight for 5 successive days) estimated by HPLC was 4565.1±1.640, 3801.4±0.341, 3433.2±0.904, 3238.5±0.906, 2938.2±0.755, 2612.1±0.611, 2024.5±0.559, 1533.6±0.636, 198.2±0.204 and 1.4±0.003 µg/kg, respectively, but after boiling they were 2842.1±0.988, 3126.9±0.885, 3219.4±0.984, 2845.5±1.013, 2474.7±0.665, 1568.3±0.900, 1321.1±0.747, 1071.9±0.733, 45.3±0.042and undetected µg/kg, respectively, while after freezing results in table (2) showed that for one month they were 3370.6±0.709, 3290.3±0.769, 3125.6±0.946, 2919.3±1.016, 2573.5±0.651, 1889.9±0.667, 1705.9±0.664, 1276.4±0.322, 60.8±0.059 and undetected µg/kg, respectively, after freezing for two months they were 1950.7±0.166, 1771.1±0.198, 1606.1±0.220, 1396.2±0.174, 1192.9±0.114, 1043.8±0.094, 871.3±0.064, 725.3±0.150, 1.7±0.003 and undetected µg/kg, respectively and after freezing for three months they were 828.6±0.322, 645.4±0.223, 539.5±0.244, 346.9±0.147, 195.2±0.089, 107.4±0.012, 87.3±0.019, 55.4±0.052, undetected and undetected µg/kg, respectively. It is clearly appeared that the time of slaughter after the last injected dose of oxytetracycline significantly affects the concentration of oxytetracycline in muscle, as slaughtering of rabbits after prolonged time of last dose of oxytetracycline decreased greatly the residual level until the concentration became very low at slaughter time 3 weeks after the last dose of oxytetracycline. These results are in agreement approximately with Rome (1991) and Villa *et al.* (2001) who concln.ded that there were significant differences among the sacrifice times (0, 24, 48 and 72 hours post- treatment) in all matrixes collected in rabbits and by increasing the slaughter time after the last dose, the oxytetracycline concentration decreases. Boiling significantly decreases oxytetracycline level in muscle till complete disappearance of oxytetracycline at slaughter time 3 weeks after the last dose of oxytetracycline. Oxytetracycline residues decreased by boiling (Nashwa, 2012). Cooking significantly affect the oxytetracycline level in turkey meat (Javedi, 2011). These results were nearly similar to previously recorded results Javedi (2011) and Nashwa (2012) and disagreed with those recorded by Mohammed (1997). Freezing caused partial degradation of oxytetracycline in relation to the slaughter time after the last dose injected and the period of freezing, as long storage of rabbit muscles at freezing temperature (-20° c) decreased the oxyteracycline residues until disappeared completely from the rabbit muscles which slaughtered 2 weeks and 3 weeks after the last dose injected and stored at -20° c for 3 months. These results were in a partial agreement with Hagsmaa (1993), Kan (1995), Mansour (2000), Abd El-Monem *et al.* (2002), Gehad (2002), Hanaa (2002) and Tamer (2012),as they concluded that tetracycline residues were degraded by freezing at -18° c to -20° c. While these results were disagreed with those obtained by Gehan (1991).

Table (3) showed that the mean values of oxytetracycline in kidney samples after slaughter time zero, one day, two days, three days, four days, five days, six days, one week, two weeks and three weeks was 35355.5±8.524, 22811.7±4.275, 15329.5±3.619, 10929.2±4.680, 7409.4±3.579, 5872.9±2.677, 5247±2.011, 2540±0.894, 965.1±0.543 and 17.7±0.014µg/kg, respectively, while after boiling the mean values of oxytetracycline was 14626.7±7.537, 10245.1±4.137, 7217.3±2.944, 7979.9±3.484, 5294.6±3.059, 3819.6±1.866, 3201±1.712, 1155.5±0.620, 510±0.364and undetected µg/kg, respectively, and table(4) showed that after freezing for one month the mean values of oxytetracycline was 26964.3±8.531, 17324.9±3.796, 10865.6±2.095, 9108.7±2.812, 6242.7±3.962, 4428.4±2.147, 4088.4±1.894, 1927.6±0.769, 720±0.516 and undetected µg/kg, respectively, after freezing for two months the mean values was 5297.8±0.614, 4196.7±0.675, 3077.2±0.162, 2806.7±0.414, 1882±0.318, 1696.6±0.377, 1384.5±0.308, 701.7±0.434, 20.3±0.021 and undetected µg/kg, respectively and after freezing for three months they were 1834.3±0.416, 1482.6±0.386, 1271.6±0.294, 1074.5±0.216, 911.7±0.308, 767.8±0.199, 466.3±0.152, 187.1±0.118, 5±0.005 and undetected µg/kg, respectively. High oxytetracycline concentrations were detected in the kidney and this is due to the kidney is the route of excretion of oxytetracycline. The level of oxytetracycline decreased with the increase of slaughter time. Nearly similar results were reported by Rome (1991) and Villa *et al.* (2001). Data illustrated in table (5) showed that the mean values of oxytetracycline after slaughter time at zero, one day, two days, three days, four days, five days, six days, one week, two weeks and three weeks was 7393.1±1.832, 6475.6±1.766, 4838.5±1.216, 4111.2±1.147, 3626.5±0.755, 3321.3±0.671, 2962.2±0.642, 2609.8±0.624, 1037.6±0.422 and 3.8±0.006µg/kg, respectively, while after boiling they were 5196.9±1.781, 4418.1±1.449, 3454.9±0.834, 3223.4±0.598, 2821.9±0.781, 2615.6±0.662, 2164.4±0.525, 1538.4±0.713, 481.7±0.267 and undetected µg/kg, respectively, and as showed in table (6) after freezing for one month they were 6545.5±2.021, 5949.1±1.327, 4196.4±1.052, 3637.3±0.874, 3356.7±0.314, 2918.6±0.715, 2408.3±0.565, 2157.7±0.655, 899.8±0.437 and undetected µg/kg, respectively, after freezing for two months they were 5512.9±1.507, 4978.9±1.161, 4353.6±0.849, 3532.2±0.881, 3096.9±0.822, 2725.9±0.711, 2261.6±0.518,

1942.6±0.648, 433±0.559 and undetected µg/kg, respectively and after freezing for three months they were 3328.5±0.875, 3112.7±0.909, 2959.9±0.799, 2581.5±0.588, 2122.8±0.420, 1908.7±0.451, 1441.4±0.303, 719.1±0.474, 262.9±0.372 and undetected µg/kg, respectively. The liver is the site of metabolism of oxytetracycline so the level of residual oxytetracycline is high in liver and begin gradually to be decreased with increasing the slaughter time after the last dose till reached a very low concentration after slaughter time 3 weeks. These results were nearly similar to those obtained by Rome (1991) and Villa *et al.* (2001). These results disagreed with those recorded by Mohammed (1997), Hanaa (2002) and Tamer (2012) that clarified that oxytetracycline completely disappeared by boiling regardless the initial concentration before boiling.

The recovery rates for oxytetracycline in rabbit meat, kidneys and livers was 102%, 92% and 86%, respectively, at concentration of 20 µg/kg of spiked samples.

It can be concluded that neither boiling nor freezing could be considered as a reliable method to get rid completely from oxytetracycline as they caused a partial degradation of oxytetracycline residues. The only solution depending on the obtained results is to delay the slaughter time till the oxytetracycline metabolized in liver and excreted from the kidneys, and this takes a withdrawal period about 3 weeks after a course of treatment 20 mg/kg body weight subcutaneously for five successive days.

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دراسات عن نضوب بقايا الأوكسي تتراسيكلين في لحوم الأرانب

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تعتبر لحوم الأرانب منتج عالي الجودة لاحتوائه على نسبة بروتين عالية ونسبة قليلة من الحموض الدهنية المشبعة والكوليسترول والصوديوم. وتعتبر الأرانب من أكثر الحيوانات عرضة للأمراض المختلفة مما يدفع أصحاب المزارع لاستخدام المضادات الحيوية المختلفة دون إشراف السلطات البيطرية ودون إعطاء الجرعات المناسبة وعدم مراعاة فترة سحب الدواء قبل تسويق هذه الأرانب مما يؤدي إلى وجود بقايا المضادات الحيوية في لحوم الأرانب ممثلة مشاكل للصحة وسلامة المستهلك ومن المشاكل الصحية التي يسببها الأوكسي تتراسيكلين الحساسية وانتشار الميكروبات المقاومة للأوكسي تتراسيكلين واضطرابات القناة الهضمية ومشاكل في العظام والأسنان عند الأطفال. يعتبر الأوكسي تتراسيكلين خاصة طويل المفعول من أكثر المضادات الحيوية استخداما لسهولة جرعته وتوافره ورخص ثمنه، لذلك أجريت هذه الدراسة لتحديد مدى نضوب الاوكسي تتراسيكلين في أنسجة الأرانب المختلفة (عضلات ، كلى وأكباد) عند ذبح الأرانب على مراحل زمنية مختلفة من اخر جرعة من هذا الدواء وقياس مدى تأثير الغليان لمدة نصف ساعة والتجميد عند -٢٠ درجة مئوية على بقايا الاوكسي تتراسيكلين وذلك باستخدام جهاز الكروماتوجرافي السائل عالي الاداء. هذا وقد أشارت النتائج إلى أن موعد الذبح يؤثر تأثيرا كبيرا على تركيز بقايا الاوكسي تتراسيكلين حيث يقل تركيز تلك البقايا في الأنسجة مع تأخير موعد الذبح. أيضا أدى الغليان إلى نقص ملحوظ في مستوى تلك البقايا ولكنه لم يسبب اختفائها كليا. يؤدى تجميد لحوم الأرانب لمدة شهر وشهرين وثلاثة أشهر إلى تقليل بقايا الاوكسي تتراسيكلين ولكن تأثيره اقل من تأثير الغليان ويزداد تأثير التجميد كلما زادت مدة التجميد. ولذلك نوصي بعدم الاعتماد على غليان وتجميد اللحوم للتخلص الكامل من بقايا الاوكسي تتراسيكلين ولكن أيضا بتأخير موعد الذبح والالتزام بفترة سحب الدواء وهي في حدود ثلاثة أسابيع للتخلص التام من بقايا الاوكسي تتراسيكلين في لحوم الأرانب.