

Levels of tetracycline residues in cattle meat, liver, and kidney from a slaughterhouse in Tabriz, Iran

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Abstract: Total of 500 samples from triceps, gluteal and diaphragm muscles, kidney, and liver, were obtained randomly from beef carcasses of a slaughterhouse in Tabriz.

We measured oxytetracycline, tetracycline, and chlortetracycline (TCs) residues by high performance liquid chromatography (HPLC) method. Seventy four percent of samples (380 samples) had detectable TCs residues. The mean amounts of oxytetracycline, tetracycline, and chlortetracycline residues in all samples were 52.2, 33.8, and 125.2 µg/kg, respectively. The mean amounts of total residues of TCs in all meat samples, triceps, gluteal and diaphragm muscles, kidney, and liver were 131.0, 163.1, 63.4, 166.7, 408.1, and 254.9 µg/kg, respectively. Five percent of kidney and liver samples and 21.7% of all samples contained residues more than the maximum residue limits (MRLs) of the World Health Organization (WHO).

This study indicates presence of different levels of tetracycline residues in the various edible tissues. Regulatory authorities should ensure proper withdrawal period before slaughtering of the animals.

Key words: Tetracycline, meat, HPLC

Tetracyclines (TCs) are broad-spectrum antibiotics, widely used in animal husbandry for both of prevention and treatment of diseases and as growth promotion. In food-producing animals, tetracyclines can be administrated orally through food or drinking water, parenterally, or by intramammary infusion. Due to enterohepatic circulation, a small amount of administrated dosage may persist in the body for a long time after administration (1). Antibiotic residues in foods can influence the bacterial composition and

the metabolic activity of the intestinal micro flora and the metabolism of endogenous compounds. Tetracyclines in meat potentially may stain teeth of young children. Allergic reactions and development of resistant strains of bacteria following the ingestion of subtherapeutic doses of antimicrobials are some of the hazardous effects (2-8). The World Health Organization (WHO) and the Food and Agriculture Organization (FAO) have set standards for acceptable daily intake (ADI) and maximum residue limits

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(MRLs) in foods. European Union (EU), USA, Canada, and some other countries have set MRLs, too. 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.

There are no reports for tetracycline levels in Iranian beef so this study is the first one. The aim of this study was to investigate the presence of oxytetracycline, tetracycline, and chlortetracycline (TCs) residues in various edible tissues (triceps, gluteal and diaphragm muscles, kidney, and liver) of cattle from a slaughterhouse in Tabriz, which is the capital city of East Azarbaijan Province and the fourth largest city of Iran.

Sampling was done by a systematic random sampling method in a slaughterhouse in Tabriz. In this method, we took the samples in the intervals of a number of carcasses dependent on the total numbers of carcasses to make the best distribution and randomization of sampling. We took 100 samples from each of the total of 5 locations containing triceps, diaphragm and gluteal muscles, kidney, and liver. We obtained minimum of 50 g from each sample and kept them in a -70 °C deep freezer (Snijders Scientific, Holland) until the time of analysis.

Procedures of sample preparation (9-14):

A- Homogenization: We cut samples to fine pieces and 5 g of each sample was homogenized and diluted with McIlvaine buffer (mixed citrate/phosphate, pH 4.1 with EDTA). We put the mixtures in a high intensity sonicator for 10 min followed by shaking for 10 min.

B- Filtration: The homogenized samples centrifuged at 10000 g and re-diluted and supernatants filtered through 0.45 µ filter (Nalgene, USA).

C- Solid Phase Extraction: Oasis HLB cartridge was used as follows:

- I. The cartridge conditioned by 3 mL of methanol and then rinsed by 2 mL of de-ionized water (11).
- II. The prepared mixtures in stage B were loaded in a Oasis HLB cartridge.
- III. We washed the cartridge with 2 mL of 5% methanol solution in water.

IV. We eluted TCs by 3 mL of HPLC grade methanol from the cartridge.

D. Evaporation and concentration: Eluted solutions (3 mL) evaporated to be concentrated by a rotary evaporator. Concentrations and dilution factors were calculated and considered in the calculation of the real amount of drug residues in the sample after analysis.

Chemicals, analytical standards of TCs, and Oasis HLB cartridge (WAT106202) were purchased from Merck (Germany), Sigma Chemical Co., and Waters (USA), respectively. HPLC performed using a Waters 717 plus auto sampler, Waters 515 HPLC pump, Waters 2487 dual absorbance detector, and Waters 474 scanning fluorescence detector.

The mobile phase was a mixture of methanol, acetonitrile, and 50 mM oxalic acid (10: 20: 70%). The first wavelength was 365 nm, and the second one was 255 nm. HPLC column was a Phenomenex Luna 5µ C18 (Torrance, CA, USA), and the flow rate was 1 mL/min. Several serial dilutions were prepared for each tetracycline to give the following concentrations: 10, 5, 2.5, 1.25, 0.1, 0.05, and 0.01 mg/kg, to use in preparing the standard curves. Figure A shows the HPLC curve of mixed standard solution of TCs 50 µg/kg concentration.

Concentrations of drugs in the samples extrapolated from the HPLC curves peak height. The detection limit for oxytetracycline, tetracycline, and chlortetracycline were 2.2, 2.7, and 7.6 µg/kg, respectively. Recovery, inter-assay in addition, intra-assay variations of the method were calculated (Table 1).

In analyzed samples, the mean amount of oxytetracycline was 52.2 µg/kg, its slope was 1804.2 µg/kg and 57% of the samples lacked measurable amount of oxytetracycline residues (Figure B). The mean oxytetracycline residues in triceps, gluteal and diaphragm muscles, kidney, and liver were 109.9, 65.0, 11.1, 31.9, and 43.0 µg/kg, respectively. The mean value of this drug residue in muscles was 62.0 µg/kg (Table 2).

In the analyzed samples, the mean amount of tetracycline was 33.8 µg/kg and 72% of samples lacked measurable amounts of tetracycline residues. The mean value of tetracycline residues in triceps, gluteal

Table 1. Recovery, inter-assay, and intra-assay variations of the method used for detection of TCs in samples.

	Oxytetracycline	Tetracycline	Chlortetracycline	Total mean
Mean recovery (%)	75.18 n = 12	72.68 n = 12	71.28 n = 12	73.04 n = 36
Intra-assay variation mean C.V.%	2.89 n = 12	3.33 n = 12	4.16 n = 12	3.46 n = 36
Inter-assay variation mean C.V.%	7.62 n = 12	7.71 n = 12	8.34 n = 12	7.89 n = 36

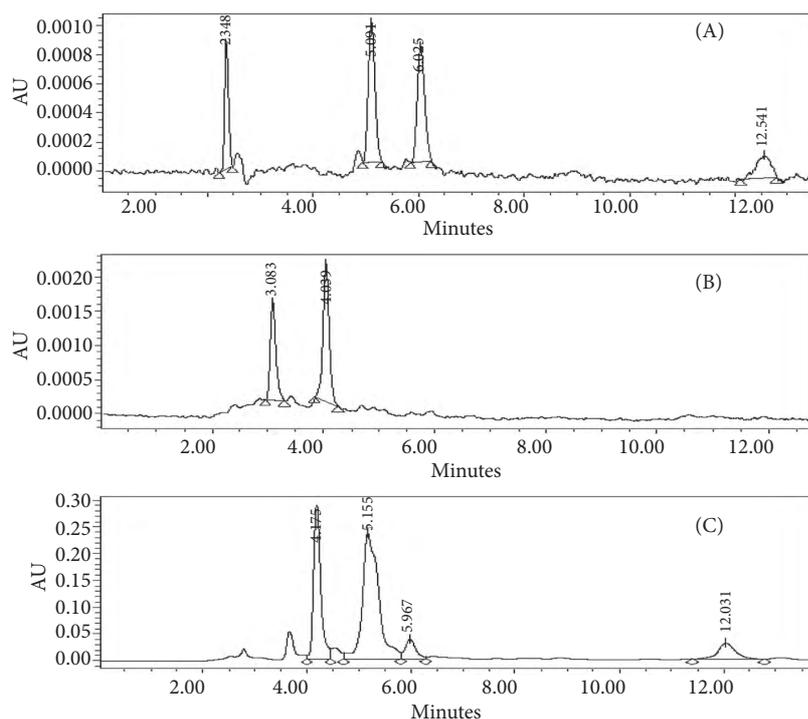
Figure. The HPLC chromatograms of (A) mixed standard solution of TCs 50 $\mu\text{g}/\text{kg}$ concentrations, (B) a meat sample without any TCs residues, and (C) a meat sample containing all 3 TCs residues.

Table 2. Mean TCs residues in meat, liver, and kidney samples.

	Oxytetracycline ($\mu\text{g}/\text{kg}$)	Tetracycline ($\mu\text{g}/\text{kg}$)	Chlortetracycline ($\mu\text{g}/\text{kg}$)	Mean of total TCs residues ($\mu\text{g}/\text{kg}$)
Triceps muscle	109.9	4.5	48.7	163.1
Gluteal muscle	65.0	48.4	53.2	166.7
Diaphragm muscle	11.1	2.8	49.5	63.4
Meat (mean of muscles)	62.0	18.7	50.5	131.0
Liver	43.0	112.9	98.9	254.9
Kidney	31.9	0.4	375.8	408.1
Total Mean	52.2	33.8	125.2	211.2

and diaphragm muscles, kidney, and liver were 4.5, 48.4, 2.8, 0.4, and 112.9 µg/kg, respectively. The mean value of this drug residue in muscles was 18.7 µg/kg (Table 2).

In analyzed samples, the mean value of chlortetracycline was 125.22 µg/kg and 50% of samples lacked measurable residues of chlortetracycline. The mean values of chlortetracycline in triceps, gluteal and diaphragm muscles, kidney, and liver were 48.7, 53.2, 49.5, 375.8, and 98.9 µg/kg, respectively. The mean value of these drug residues in muscles was 50.5 µg/kg (Table 2).

The mean value of total residues of the TCs in all samples was 211.2 µg/kg. The mean value of total TCs residues in triceps, gluteal and diaphragm muscles, kidney and liver were 163.1, 166.7, 63.4, 408.1, and 254.9 µg/kg, respectively (Table 2). The mean value of total residues in the 3 muscles was calculated 131.0 µg/kg and 24% of samples lacked measurable amounts of the mentioned 3 drug residues. Figure 1C shows the HPLC curve corresponding to a meat sample containing all 3 TC residues.

The mean value of oxytetracycline residues in analyzed samples was 52.2 µg/kg, less than the maximum residue limit (MRL) in the European Union (100 µg/kg), World Health Organization, U.S.A, Japan (200 µg/kg), and Australia (250 µg/kg). Three percent of the samples contained oxytetracycline more than the MRL in Australia (250 µg/kg). The mean amounts of oxytetracycline in triceps muscles was 9.9 µg/kg more than the MRL in the European Union and 8.3% of meat samples (muscles) contained oxytetracycline more than the MRL in the European Union. Five percent of kidney and liver samples contained oxytetracycline more than the MRL of codex, U.S.A, and Japan (15-18).

The mean tetracycline residues in the analyzed samples was 33.8 µg/kg, less than MRL of European Union, Australia (100 µg/kg), codex, U.S.A., Japan (200 µg/kg), and Canada (250 µg/kg). Four percent of samples contained drug more than the MRL of Australia (100 µg/kg). The mean value of tetracycline residues in liver was 12.9 µg/kg more than the MRL in Australia. Five percent of liver samples and 1.6% of meat (muscle) samples, compared to codex, European Union, U.S.A., Canada, Australia, and Japan

standards, contained more tetracycline residues (15-18).

The mean value of chlortetracycline in the analyzed samples was 125.2 µg/kg, less than the MRL of codex, U.S.A., and Japan (200 µg/kg), but exceeded the MRL of the European Union and Australia (100 µg/kg). Mean concentrations of chlortetracycline residues in 15% of the meat samples exceeded the MRLs of the European Union, Australia, and Canada, and 8.3% of the samples contained more drug residues than MRLs of codex, U.S.A., and Japan. Mean concentrations of chlortetracycline residues in kidney exceeded the MRLs of America, Japan, Australia, and Canada, but it was less than MRLs of codex (1200 µg/kg) and European Union (600 µg/kg). Chlortetracycline residues in 75% of kidney samples and 35% of liver samples exceeded the MRLs of Canada and Australia. These drug residues in 50% of kidney and 20% of liver samples exceeded the MRLs of Japan and U.S.A., and 15% of kidney and 10% of liver samples contained more drug residues than codex standard (15-18).

The mean concentration of TC total residues in the analyzed meat samples was 131.0 µg/kg, less than the MRL of codex, U.S.A, and Japan (200 ppb) but exceeded the MRL of the European Union (100 ppb).

The mean values of TC total residues in triceps and gluteal muscles were more than the diaphragm muscle. This can be due to vicinity of sampling and drug injection points in comparison with the diaphragm muscle.

Drug concentrations in 21.7% of the meat samples exceeded the MRL of the European Union and, in 13.3% of them, they were more than the MRLs of codex, U.S.A., and Japan. The mean value of drug residues in kidney exceeded the MRLs of U.S.A. and Japan but were less than the MRLs of codex (1200 ppb) and the European Union (600 ppb). In 55% of kidney samples, drug concentrations exceeded the U.S.A. and Japan MRLs. Fifteen and 5% of liver samples had drug residues more than the European Union and codex MRLs, respectively. Forty-five percent of liver samples, compared to the U.S.A. and Japan MRLs, and 25%, compared to the European Union MRL (300 ppb), and 5% compared to the codex MRL contained more TC residues.

The present study indicates the presence of tetracyclines residues in edible tissues from a slaughterhouse in Tabriz. Most samples contained more intended drug residues than the MRLs. There is not sufficient number of study in Iran about drug residues in edible tissues. Abovementioned amounts of drug residues can accomplish drug resistance in consumers and perhaps in some cases, can have digestive and allergic effects. It would also change organoleptic specifications in some meat samples.

Therefore, edible tissues of cows in Tabriz do not have desired conditions because of the presence of

tetracycline residues more than the MRLs. Further studies are necessary to evaluate other drug residues in referred edible tissues and to evaluate the hazardous of these residues in relation with daily intakes and other related factors.

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