Table 5. *Number of OTC containing samples exceeding the MRL.*

Type of Samples	No. of positive samples	No. of samples above MRL	% of samples above MRL*	MRL µg/g
Breast	46	4	8.69	0.2
Thigh	120	19	15.83	0.2
Liver	160	72	45	0.6
Kidney	160	82	51.25	1.2

*The percentages were calculated according to the number of positive samples **(WHO/FAO, 2010).**

Table 6. *Statistical analytical results of TC concentration µg/g in poultry samples.*

Type of Samples	No. of samples examined	No. of positive samples	Percentage of positive samples	Conc. of TC µg/g		
				Min.	Max.	Mean ± SD
Breast	160 samples each	8	5.0	0.13	0.80	0.3905 ± 0.2633
Thigh		20	12.5	0.15	0.50	0.2720 ± 0.1281
Liver		21	13.1	0.13	0.26	0.1734 ± 0.0327
Kidney		8	5.0	0.14	0.32	0.2107 ± 0.0811

Table 7. *Number of TC containing samples exceeding the MRL.*

Type of Samples	No. of positive samples	No. of samples above MRL	(%) of samples above MRL*	MRL (µg/g)
Breast	8	4	50	0.2
Thigh	20	10	50	0.2
Liver	21	0	0	0.6
Kidney	8	0	0	1.2

There are agreement with the method developed by Biswas et al. [14] as they use the same mobile phase but with relatively low pH=1.6 and low flow rate 0.6 mL/min using an Rp-C8 phenomenex column at 35°C room temperature at 355 nm. They found that the tetracyclines compound at high flow rate could not be separated from the matrix interference due to reduce sensitivity of column. On the other hand, a mobile phase containing oxalic acid avoid forming chelate complex on RP-columns and interact with silanols and avoid also tail peaking with good separation for tetracyclines [10,15,16].

Moreover, to insure validation of analytical method and verify the absence of potential interfering substances around the retention time of both OTC and TC compounds, a number of blank and spiked chicken meat samples were analyzed in order to assess the specificity of the method. The results showed that, no interference was observed in the region where OTC and TC were eluted as shown in Figures 3-5.

Also, linear plots were obtained for both OTC and TC and the equation for the calibration curve was Y=7609.9 × -377.67, Y= 9032.2 × - 702.01 and the correlation coefficient (R^2) equaled 0.9985 and 0.9979 for OTC and TC, respectively as showed in Figure 6.

Recovery trials

Table 2 summarizes the mean recovery percentage of spiked OTC samples at different concentrations of 0.1, 0.2, 1.0 and 2.0 µg/g. Chicken muscle samples showed 84.22, 86.11, 86.95 and 90.62 recovery percentages, respectively with an average of 86.97; while liver samples showed recovery of 80.22%, 84.54%, 82.32 and 86.50%, respectively with an average of 83.39%. Kidney samples had recovery of 82.36%, 85.04%, 80.91% and 87.30%, respectively with an average of 83.90%.

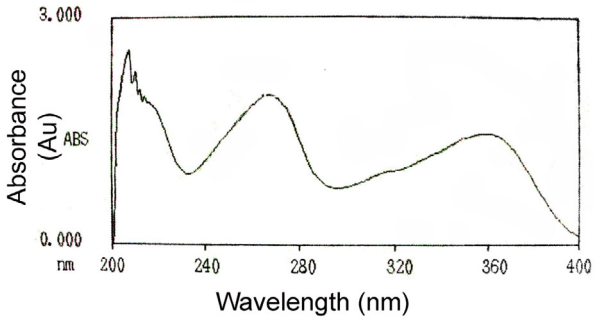

Figure 1*. UV scans of Tetracyclines standard 0.01molarity (200 – 400 nm) using Spectrophotometer.*

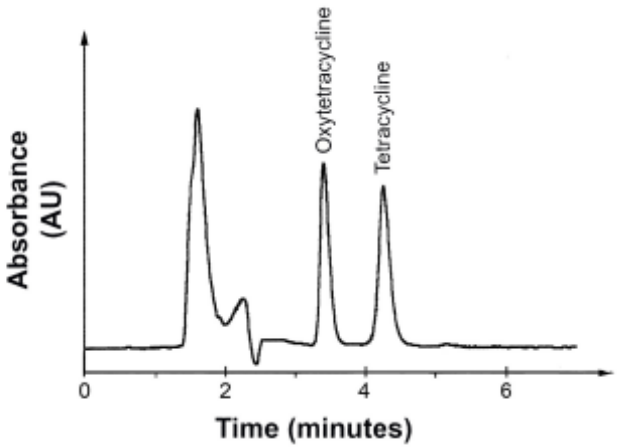

Figure 2*. Chromatogram obtained from standard solution of OTC and TC.*

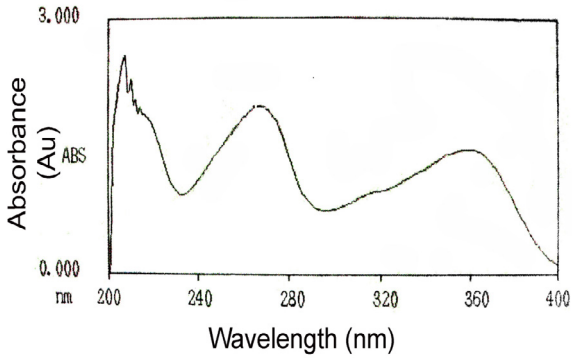

Figure 3. *Chromatogram obtained from spiked muscle samples of OTC and TC.*

Regarding recovery percentages of TC spiked samples, Table 3 summarizes the recovery percentage at the same spiking levels for OTC which recorded 81.24%, 77.76%, 73.66 and 77.58% for muscle samples, respectively with an average of 77.56%. Liver spiked samples had recovery percentages of 79.82%, 75.58%, 68.31% and 74.82%, respectively with an average of 74.63%; while kidney spiked samples showed recovery percentages of 80.55%, 74.17%, 69.78% and 73.40%, respectively with an average of 72.45%. The results which declared that the method was highly sensitive for OTC than TC.

A recovery from 50% to 80% for tetracyclines and their epimers from spiked chicken muscle samples with the same mobile phase recorded by Gajda and Posyniak [16] while, higher recovery percentage (more than 91%) was recorded [3].

Incidence of Tetracycline's veterinary drug residues in the examined samples

Incidence of OTC drug residues in the examined samples The residue concentrations of OTC were detected in 46 (28.75%) of breast muscle samples from 0.08 to 0.35 µg/g with a mean value of 0.1296 ± 0.0532. Four samples (8.69%) showed residues above the MRL as recorded [5], while the residual concentrations were detected in 120 (75%) of thigh muscle samples with quantities ranged from 0.08 to 0.32 µg/g and with a mean value 0.1618 ± 0.0532. Nineteen samples (15.83%) showed residue concentration above the MRL as set [5] (Tables 4 and 5).

All liver and kidney samples (100%) examined contained OTC. Liver samples contained OTC in quantities ranged from 0.14 to 3.36 µg/g and a mean value of 0.7223 ± 0.5176 µg/g and in quantities ranged from 0.20 to 3.83 µg/g, with a mean value 1.3211 µg/g ± 0.7850 for kidney samples. Seventy two (45%) liver samples showed residual concentration above the MRL while eighty two (51.25%) kidney samples showed residue levels above the MRL as set [5] (Tables 4 and 5).

Our findings proved that OTC percent was higher in liver and kidney samples than breast and thigh muscle samples. The results were not unusual since the liver and the kidney are the major storage and excretory organs for tetracycline's [2] and due to general increases in the abuse of OTC antibiotic drugs in some farms where withdrawal period is not observed before slaughtering of the chicken. Moreover, Prescott and Baggot [17] stated that tetracyclines were undergo extensive entrohepatic circulation which lead to prolongation of their elimination half-lifes; thus persisting in the body for long time after cessation of drug administration. Long withholding periods for oral administration of tetracyclines (up to 21 days depending on indication and type of drug) have been recommended in the USA [18].

Nearly similar results was recorded [3] who examined 100 chicken samples and found that 57.1% of the muscles were positive for OTC residues while they found lower concentrations, 23.1% and 66.7% in liver and kidney samples respectively.

Incidence of TC residues in the examined samples

The residual concentrations of TC were detected in 8(5%) of breast muscle in quantities ranged from 0.13 to 0.80 µg/g and a mean value of 0.3905 ± 0.2633. Four (50%) of the examined samples showed residues above the MRL (Tables 6 and 7). The wide variation in the quantities of residues detected in the examined breast muscle samples may be due to differences in the route of the drug administration for treatment purposes and/or through drinking water or by addition to the animal feed as growth promoters and these indicates differences in animal husbandry practices from different farms.

The residual concentrations of TC was detected in 20 (12.5%) of thigh muscle samples with a concentration ranged from 0.15 to 0.50 µg/g and a mean value of 0.2720 ± 0.1281. Ten samples (50%) of the examined samples showed residues above the MRL (Tables 6 and 7).

In case of liver samples the residual concentrations was detected in 21 (13.1%) with a concentration ranged from 0.13 to 0.26 µg/g and a mean value 0.1734 ± 0.0327. Moreover, the residual concentration level was detected in 8 (5%) of the examined kidney samples in quantities ranged from 0.14 to 0.32 µg/g, and a mean value of 0.2107 ± 0.0811. None of the examined liver and kidney samples had residues of TC drug above the MRL (Tables 6 and 7). In spite of, tetracycline residues were detected with a high percentage in the liver samples than in the kidney

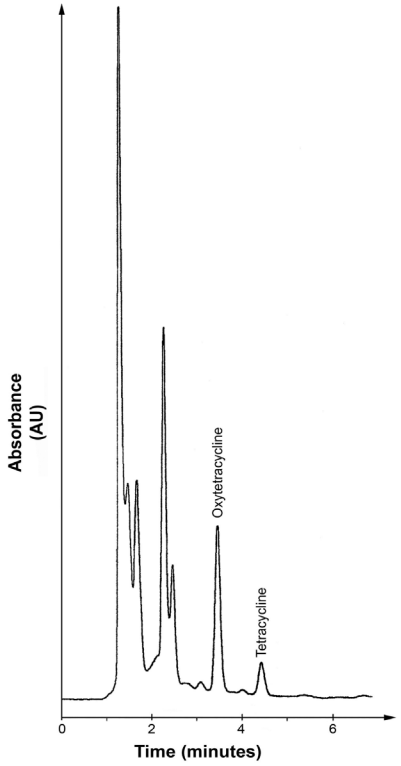

Figure 4. *Chromatogram obtained from spiked liver samples of OTC and TC.*

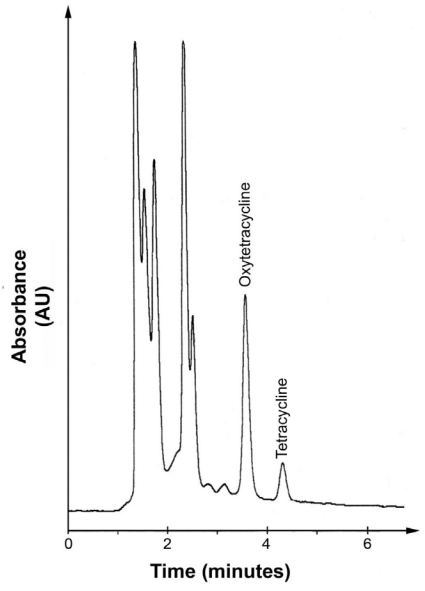

Figure 5*. Chromatogram obtained from spiked kidney samples of OTC and TC.*

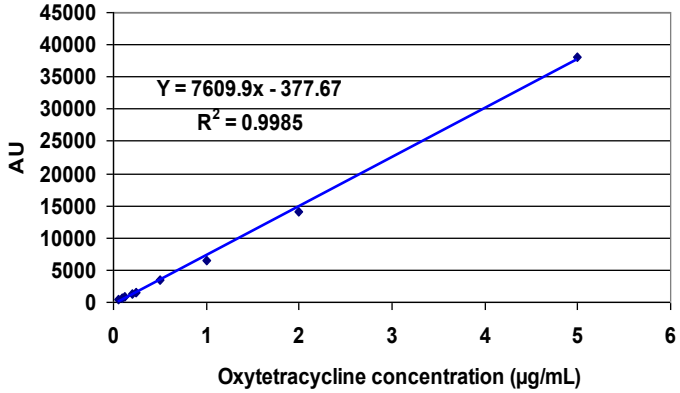

Figure 6. *A calibration curve of OTC hydrochloride.*

samples. This could suggest that, these samples were taken at the time when drug were metabolized in the liver not yet at the stage of clearance by the kidney. Therefore, antibiotics were most often administered close to the time of slaughter.

Similar results was reported by Abdulsalam et al. [19] who recorded that tetracycline residues were found more in cattle liver (44%) than its kidney samples (12%). Muriuki et al. [20] found that the percentage of TC residues in chicken samples were 24%, 14% and 7.6% in liver, kidney and muscle samples, respectively.

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