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Determination and Validation of Tetracycline Residues in Poultry using High Performance Liquid Chromatography - Diode Array Detector Technique

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Abstract

The aim of this work was to separate and determine the trace tetracycline residues in poultry chest, thigh and liver using High Performance Liquid Chromatography - Diode Array Detector (HPLC-DAD), with a mobile phase mixture consisting of acetonitrile: methanol: oxalic acid (0.01M) (25:15:60) and chromatographic column C_8. The study was included 32 live poultries, which were received a chest injection of 1m of tetracycline standard solutions. Over four successive days, poultries were slaughter for analysis.

The injection with 10×10^3 ppb of tetracycline showed that the traces of tetracycline residues exceeded the maximum residue limit (MRL = 200 ppb) in the thigh and chest meat in the 1st day and over first and second day of slay respectively. The traces of tetracycline have exceeded the value of (MRL = 600 ppb) in the liver over third and fourth day of slay.

Limit of detection was LOD = 0.451 ppb, limit of quantification LOQ was 1.502 ppb, and recovery% of tetracycline at a concentration of 200.0 ppb was (88.966 - 91.055%) for Poultry chest, (84.623 - 87.667%) for thigh and of around (82.198 - 83.688%) for liver with a percentage relative standard deviations (RSD%) of < 1 %.

Keywords: Tetracycline, HPLC-DAD, poultry chest, thigh and liver, validation of analytical method.

الخلاصة

يهدف البحث إلى فصل وتحديد نزر مركب التتراسيكلين المتبقي في صدر الدجاج وفخذه وكبده باستخدام تقانة الكروماتوغرافيا السائلة عالية الأداء المزودة بكاشف المصفوفة الديودية الضوئية وطور متحرك لمزيج من ميثانول: أسيتونتريل :حامض الاوكزاليك بتركيز 0.01M وبنسبة مزج حجميه (25:15:60) باستخدام العمود الكروماتوغرافي C_8.

أُجريت الدراسة على 32 عينة من الدجاج الحي، إذ تم ذُبحها بعد متابعة يومية لمدة أربعة أيام متتالية وذلك بعد حقنها في منطقة الصدر بـ 1 ml من محلول مُركب التتراسيكلين. بينت النتائج ان نزر مُركب التتراسيكلين المُتبقي عن حقن 10.0×10^3ppb قد تجاوز قيمة الحد الأعلى المسموح به (MRL = 200 ppb ، وفق هيئة الدستور الغذائي الأوروبي) في منطقة الفخذ خلال اليوم الأول وفي منطقة الصدر خلال اليومين الأول والثاني من الذبح. اما قيمة نزر مركب التتراسايكلين المتبقي في لحم الكبد فقد تجاوز قيمة الحد الأعلى المسموح به MRL = 600 ppb خلال اليومين الثالث والرابع من الذبح. بلغ حد الكشف LOD = 0.451 ppb، وحد التحديد الكمي LOQ = 1.502 ppb، ومتوسط النسب المئوية لاسترداد مركب التتراسيكلين بتركيز 200.0ppb لـ 20 عينة من صدر الدجاج وفخذه وكبده ما بين (88.966 - 91.055%)، و(87.667% - 84.623)، و (83.688% - 82.198) على التوالي وبانحراف معياري نسبي مئوي RSD% لم يتجاوز 1%.

Introduction

In modern agricultural practice, veterinary drugs are used in a large scale and administered as feed additives or added to the drinking water, in order to prevent the outbreak of diseases [1]. They also are used for therapeutic, and growth promotion purposes [2]. Various human activities such as industrial and domestic wastes and agricultural inputs cause contaminants to enter aquatic environments [3][4]. Increasing the antibiotic-resistance leads to problems in the treatment of infectious diseases worldwide [4]. Tetracyclines are widely used in animal

husbandry as veterinary drugs, due to their broad-spectrum activity and low cost [5]. The Codex Alimentarius Commission of the FAO/WHO has reported that the maximum residue limits MRLs of tetracycline residues are 200 ppb in muscle, and 600 ppb in liver [6]. Multi-residue detection methods, which simultaneously determine more than one class of veterinary drugs in any matrix, are still limited and are largely confined to liquid chromatography–mass spectrometry (LC–MS) methods. Diode array detector (DAD) as a detector for high performance liquid chromatographic (HPLC) has proved to be a powerful tool for determining and identifying compounds, as it makes possible the on-line acquisition of their UV spectra. In addition, most of the mentioned methods above are used for one class of antibiotics [7]. Figure 1 showed the chemical structure of the tetracycline hydrochloride.

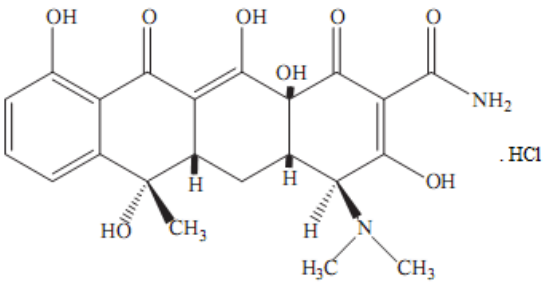

Figure 1: Chemical structure of the Tetracycline hydrochloride.

The aim of this study was to detect the trace of tetracycline residues in poultry chest, thigh and liver after few days of injection different concentrations of tetracycline. HPLC–DAD and the chromatographic column C_8 were used to determine the traces amount.

Validation of the analytical method was achieved to check the analytical purpose of the method is achieved, which is obtaining analytical results with an acceptable uncertainty level or a good confidence level [8]. This validation was followed by identifying each of the Limit of Detection LOD, Limit of quantification LOQ, and recovery.

Definition of validation parameters

Limit of detection (LOD) is the lowest amount of an analyte in a sample, which can be detected (but not necessarily quantified) as an exact value of the signal to noise ratio is 3 [8].

Limit of quantification (LOQ) is the lowest amount that can be analysed within acceptable precision and accuracy when the signal to noise ratio is 10 [8].

*Recovery (%)***:** The accuracy of the method was assessed by recovery test. The recovery of an analytical method is a parameter to measure the efficiency of the method used in the analytes extraction process [9].

Experimental

Materials and reagents

Tetracycline hydrochloride (92.6%), Methanol, MeOH (99.8%), Acetonitrile, ACN (99.8%), Phosphoric Acid, H_3PO_4 (99.5%), Disodium Hydrogen Phosphate anhydrate Na_2HPO_4 (98%), were obtained from Merck, Darmstadt, Germany. Oxalic acid, $H_2C_2O_4.2H_2O$ (98%) was obtained from Qualikems-India. Citric acid, $C_6H_8O_7.H_2O$ (99.5%) was obtained from Prolabo-CE. Ethylenediaminetetraacetate EDTA (99.5%) was obtained from Poch SA (Poland) and de-ionized water was used for preparing all the aqueous solutions. All chemicals used were of analytical grade.

Apparatus and Tools

Filter paper obtained from Zelpa, Belgium. Oasis HLB SPE cartridges (500 mg, 5 m*l*). Chromatographic Column Agilent ZORBAX Eclipse XDB C_8(250 x 4.6mm id., 5µm). Digital Analytical Balance Weighing, 200/0.0000g±0.1 mg from Genius, Germany. Homogenizers Dispersers Ultra turrax, IKA T18 basic. Rotavoper from BUCHI, Japan. Ultrasonic water – bath, Transsonice T700. pH, Crison. Centrifuge 5000 rpm, Tomy LC-100. Solid phase extraction apparatus, Supelco, USA.

Solvents and extracted samples were filtered with Teflon and Nylon filter 0.45 µm (Albet, Germany). The chromatographic system was supplied with a diode array detector, from Agilent Technologies, Palo Alto, CA, USA,

Preparation of Solutions

- Mobile phase: A ternary mixture of oxalic acid (0.01M), acetonitrile and methanol was prepared in a volumetric ratio (25:15:60 %).
- EDTA-McIlvaine buffer solution (pH=2.6), was freshly prepared by dissolving 11.80 g of citric acid monohydrate $C_6H_8O_7 .H_2O$, 13.72 g of anhydrous Na_2HPO_4 and 33.62 g of Na_2-EDTA in one liter of de-distilled water .The mixture was kept in a dark-color bottle at -20 °C until further use [10].
- A series of standard solutions of tetracycline (1.0-10 $x10^3$ ppb) were prepared in order to determine the trace residue of the antibiotic using HPLC-DAD technique.

HPLC–DAD equipment and conditions

Chromatographic column C_8, mobile phase for HPLC was prepared by mixing a solution of methanol: acetonitrile: oxalic acid (0.01M) (25:15:60 v/v).

The flow rate was 1mL/min, and the column temperature was 40°C. The injection volume was 20 µL and the compounds studied eluted within 10 min, the wavelength was 269 nm, and the retention time was R_t: 4.169 min. Table (1) Illustrated the analytical conditions of HPLC-DAD analysis of Tetracycline [11].

Table 1: Analytical conditions for HPLC-DAD analysis of Tetracycline.

mobile phase	Acetonitrile: methanol: oxalic acid (0.01M) (25:15:60)%
column temperature	40 °C
Wavelengths	$\lambda_{.max}$ = 269 nm
flow rate	1 ml/min
Retention time	R_t = 4.169 min
Chromatography column	C_8 (250x4.6mm, id., 5µm)
Injection volume	20µℓ

Sampling

Thirty two poultry birds of 21 days old were selected, which were placed in an antibiotic-free nutrition system for nine days. Birds were fed by milled corn grains until reaching the suitable age for the study (30 days). The poultry birds were four sets. Two blank sets used to determine all concentrations of the trace residues and other two sets of birds were used to determine each concentration. The concentration of the injected tetracycline was (0.10-1.0-10.0) $x10^3$ ppb. All sets of poultry (except the blanks sets) were injected with the approved concentrations of the antibiotic at the chest within one day and at the same time.

Eight birds were slaughtered (two blank birds for all concentrations and two tested birds for each concentration every day and over a course of four days. Skin and fat layers were removed to collect samples from the chest, thigh, and liver of each bird.

Sample preparation

The studied samples were prepared according to the following extraction conditions [12]:

-two g of chest, thigh or liver was weighed.

-the sample was milled and homogenized using a laboratory milling machine. During milling 2 mL of the solvent was used for extraction and (McIlvain–EDTA at pH=2.6) was added and homogenized with 30 mL of the same solvent for one minute.

-the mixture was processed using an ultrasonic wave at 30 °C for half an hour, then the resulted sample was centrifuged for 15 minutes.

- after 15 min, the liquid layer was separated and filtered.

- the extracted (protein –free) liquid layer was passed onto a solid phase extraction cartridge (SPE-Oasis HLB), in order to isolate the antibiotic.

-tetracycline was eluted from the extraction cartridge with 3.5 mL of methanol.

the final extract was then passed through a 0.45 µm size filter which becomes ready for injection into (HPLC–DAD).

Residue Percentage

The residue percentages of tetracycline compound (Res. %) in poultry samples were quantified through [replicating the injection of extraction products of four independent poultry chest, thigh and liver samples for each level. The average percentage of the residue of

tetracycline compound in the extraction output of the four samples was calculated by means of the following relationship:

$$\text{Residue percentage \%} = \frac{\text{Residue average concentration (ppb)}}{\text{Injected solution concentration (ppb)}} \times 100$$

Preparation of Standard Solutions

- The standard stock solution: 10.820 mg of tetracycline hydrochloride was dissolved in methanol up to 10.0 mL. The final dissolution process was completed in an ultrasonic water bath for five minutes, then the solution was diluted with methanol, to obtain a standard solution $1.0x10^6$ ppb of tetracycline. Data was labeled before covering the flask with an aluminum foil and stored at -20 °C for later use.
- The middle standard solution: 1.0 mL of the standard stock solution was transferred to a standard flask of 100 mL then diluted by methanol to the graded mark, and a middle standard solution of 10.0×10^3 ppb was obtained. Data was labeled before covering the flask with an aluminum foil and stored at -20 °C for preparing a series of standard solutions.
- Standard Solutions: from the middle standard solution, a series of standard solutions of concentrations: 1.0, 5.0, 10.0, 50.0, 100.0, 500.0, 1000.0, 1500.0, 2000.0, 2500.0, 5000.0, 7500.0, 10000.0ppb were prepared.

Calibration Curve

The linear calibration curve of tetracycline was studied using the external standard method within a concentration range of 1.0-10000.0 ppb using HPLC– DAD in order to determine the trace tetracycline residues in poultry chest, thigh and liver samples according to the analytical conditions stated in Table (1). A volume of 20 µL of each concentration was injected for four consequential times. The corresponding calibration curve based on the relationship between peak area and concentration is illustrated in Figure (2).

Table (2) and Figure (2) showed excellent linearities of the three different ranges of the calibration curves for tetracycline with three very high values of correlation coefficient R^2=0.999960, R^2= 0.999787, R^2 = 0.999783 at concentration ranges of 1.0-100.0ppb, 50.0-2000.0ppb, 1000.0-10000.0ppb respectively.

Table 2: Peak area averages of tetracycline calibration curves range concentration between (1.0-10000.0ppb)

Con. (ppb)	*Peak area (mAu.min)	RSD%**	CL***
1.0	0.062	0.828	0.001
5.0	0.324	0.399	0.002
10.0	0.606	0.233	0.002
50.0	3.215	0.103	0.005
100.0	6.420	0.034	0.003
500.0	32.100	0.062	0. 028
1000.0	65.980	0.114	0.104
1500.0	98.320	0.004	0.005
2000.0	128.731	0.004	0.006
2500	151.480	0.005	0.011
5000	303.041	0.002	0.007

*: The average of peak area in four replicates **: percentage relative standard deviation. ***: Confidence limit at 95% confidence level. Confidence level is given by $\overline{X} \pm \frac{t \times SD}{\sqrt{n}}$.

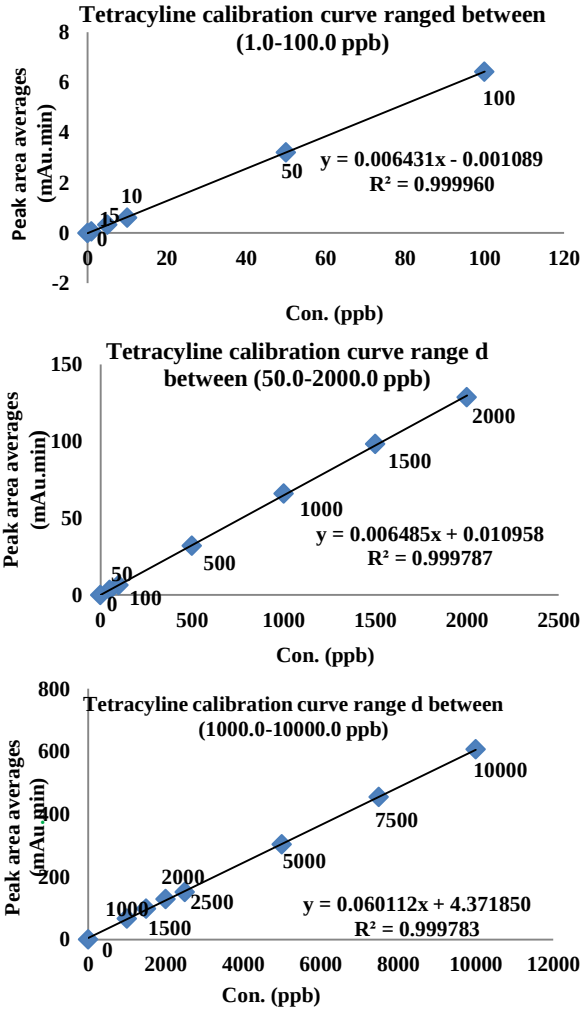

Figure 2: Three calibration curves of tetracycline concentration range between (1.0-10000.0 ppb).

Results and Discussion

Selected series of live poultry was injected in the chest with 1 mL of different concentrations of tetracycline (0.10, 1.0 and 10.0) x 10^3 ppb. Poultries were slaughtered over a period of one to four days after injection. Traces of the tetracycline residue in poultry samples were determined according to the optimum analytical conditions in Table 1. Tables (3, 4, 5) illustrated the average concentrations of the tetracycline residue in two independent poultry samples of the chest, thigh and liver. Table 3 illustrated the reduction in tetracycline residue in thigh as a function of time. Any trace of the tetracycline residue was not detected in the fourth day of the test, although 0.1x 10^3 ppb was injected. The reduction of tetracycline may due to the metabolism process in poultry over four days. The average of the percentage ratio of the tetracycline residue in both samples of thigh meat was reduced over four days. It was 1.448, 1.910, 1.997 % in the first day but reduced to 0, 0.053, 0.056 % in the fourth day, which were in conformity with concentration (0.10, 1.0, 10.0) $\times 10^3$ respectively.

Table 4 showed an increased tetracycline residue in poultry chest of all poultry samples compared to the thigh under the same conditions. On the other hand, tetracycline residue in the chest of poultry was reduced over time, (from the first day to the fourth day). The average of the percentage ratio of tetracycline residues decreased from 5.089, 6.675, 7.032% in the first day to 0.275, 0.354, 0.375% in day four of the experiment which were in conformity with concentration (0.10, 1.0, 10.0) $\times 10^3$ respectively.

Table 3: Average concentration of tetracycline residues (ppb) in the thigh meat of each independent poultry individual.

day	**0.10×10³ ppb**		**1.0×10³ ppb**		**10.0×10³ ppb**	
	Sample (1)	Sample (2)	Sample (1)	Sample (2)	Sample (1)	Sample (2)
	Con*±RSD%	Con*±RSD%	Con*±RSD%	Con*±RSD%	Con*±RSD%	Con*±RSD%
	$\overline{Con.}$ %**	$\overline{Con.}$ %**	$\overline{Con.}$ %**	$\overline{Con.}$ %**	$\overline{Con.}$ %**	$\overline{Con.}$ %**
	X± CL***		X± CL***		X± CL***	
1	1.489±4.367	1.406±7.908	19.648±1.756	18.549±1.519	201.037[a] ±1.137	198.416±1.041
	1.489	1.406	1.965	1.855	2.010	1.984
	1.489±0.103	1.406±0.177	19.648±0.549	18.549±0.448	201.037±3.363	198.416±3.287
2	0.511±5.228	0.498±6.782	6.749±6.728	6.330±8.488	69.917±2.292	70.104±2.846
	0.511	0.498	0.675	0.633	0.699	0.701
	0.511±0.042	0.498±0.054	6.749±0.722	6.330±0.855	69.917±2.550	70.104±3.174
3	0.207±5.003	0.200±5.957	2.707±6.873	2.525±6.243	28.284±4.646	27.659±5.593
	0.207	0.200	0.271	0.253	0.283	0.277
	0.207±0.016	0.200±0.019	2.707±0.296	2.525±0.341	28.284±2.091	27.659±2.461
4	nd.	nd.	0.515±9.896	0.544±6.975	5.620±7.411	5.622±2.140
			0.052	0.054	0.056	0.056
			0.369±0.060	0.365±0.096	5.620±0.281	5.622±0.663

n = 4: Number of injection times of each extract. *: The average of Tetracycline residues in four replicated extractions of thigh meat from one independent poultry individual. **: The average of the percentage of Tetracycline residues in four replicates drawn from the thigh meat of one independent poultry individual. ***: Confidence limit at 95% confidence level. Confidence level is given by $\bar{X} \pm \frac{t \times SD}{\sqrt{n}}$. a: Average of trace concentration of Tetracycline residues higher than the MRL value.

Table 4: Average concentration of tetracycline residues (ppb) in the chest meat of each independent poultry individual.

day	**0.10×10³ ppb**		**1.0×10³ ppb**		**10.0×10³ ppb**	
	Sample (1)	Sample (2)	Sample (1)	Sample (2)	Sample (1)	Sample (2)
	Con*±RSD%	Con*±RSD%	Con*±RSD%	Con*±RSD%	Con*±RSD%	Con*±RSD%
	$\overline{Con.}$ %**	$\overline{Con.}$ %**	$\overline{Con.}$ %**	$\overline{Con.}$ %**	$\overline{Con.}$ %**	$\overline{Con.}$ %**
	X± CL***		X± CL***		X± CL***	
1	5.220±5.886	4.959±4.868	68.456±4.031	65.036±2.654	711.379[a] ±0.251	695.029[a] ±0.356
	5.220	4.959	6.846	6.504	7.114	6.950
	5.220±0.489	4.959±0.672	68.456±4.390	65.036±2.746	711.379±2.845	695.029±3.939
2	2.793±5.833	2.679±7.772	36.974±3.034	34.989±2.654	374.003[a] ±4.778	383.594[a] ±0.819
	2.793	2.679	3.697	3.499	3.740	3.836
	2.793±0.259	2.679±0.331	36.974±1.785	34.989±1.477	374.003±2.210	373.488±5.001
3	1.361±2.991	1.360±4.713	17.177±2.186	18.153±2.985	184.512±1.261	188.953±1.475
	1.361	1.360	1.718	1.815	1.845	1.890
	1.361±0.102	1.360±0.065	17.177±0.862	18.153±0.597	184.512±3.703	188.953±4.433
4	0.272±4.617	0.277±3.279	3.651±3.418	3.423±8.271	37.578±1.909	37.319±4.116
	0.272	0.277	0.365	0.342	0.376	0.373
	0.272±0.020	0.277±0.014	3.651±0.199	3.423±0.450	37.578±1.141	37.319±2.444

n = 4: Number of injection times of each extract. *: The average of Tetracycline residues in four replicated extractions of chest meat from one independent poultry individual. **: The average of the percentage of Tetracycline residues in four replicates drawn from the chest meat of one independent poultry individual. ***: Confidence limit at 95% confidence level. a: Average of trace concentration of Tetracycline residues higher than the MRL value.

Table 5 showed the approximated trace concentrations of the tetracycline residue in the liver of each poultry compared to its residue in the thigh. When these values compared to the residue in the chest meat of the same poultry, a decrease in the first day of experiment as a function of time was detected.

Table 5: Average concentration of Tetracycline residues (ppb) in the liver of each independent poultry individual.

day	0.10×10^3 ppb		1.0×10^3 ppb		10.0×10^3 ppb	
	Sample (1)	Sample (2)	Sample (1)	Sample (2)	Sample (1)	Sample (2)
	Con*±RSD%	Con*±RSD%	Con*±RSD%	Con*±RSD%	Con*±RSD%	Con*±RSD%
	$\overline{Con.}$ %**	$\overline{Con.}$ %**	$\overline{Con.}$ %**	$\overline{Con.}$ %**	$\overline{Con.}$ %**	$\overline{Con.}$ %**
	X± CL***		X± CL***		X± CL***	
1	1.236±4.753	1.243±3.086	16.710±2.180	15.591±2.417	267.055±1.117	271.060±2.879
	1.236	1.243	1.671	1.559	2.671	2.711
	1.236±0.093	1.243±0.061	16.710±0.579	15.591±0.600	267.055±2.969	271.060±2.493
2	2.581±6.113	2.627±2.916	33.224±1.137	34.928±2.496	572.994±0.779	564.078±0.747
	2.581	2.627	3.322	3.493	3.740	5.641
	2.581±0.251	2.627±0.122	33.224±0.760	34.928±1.387	572.994±7.101	564.078±6.706
3	3.006±6.309	3.073±2.916	38.761±1.699	40.941±2.279	669.502[a]±0.625	674.361[a]±0.277
	3.006	3.073	3.876	4.094	6.695	6.744
	3.006±0.348	3.073±0.165	38.761±1.210	40.941±1.714	669.502±7.688	674.361±3.428
4	4.033±4.796	4.089±2.870	52.399±0.941	54.365±2.670	889.834[a]±0.490	897.125[a]±0.282
	4.033	4.089	5.240	5.437	8.898	8.971
	4.033±0.306	4.089±0.187	52.399±0.785	54.365±2.309	889.834±6.935	897.125±4.021

n = 4: Number of injection times of each extract. *: The average of Tetracycline residues in four replicated extractions of liver meat from one independent poultry individual. **: The average of the percentage of Tetracycline residues in four replicates drawn from the liver meat of one independent poultry individual. ***: Confidence limit at 95% confidence level. a: Average of trace concentration of Tetracycline residues higher than the MRL value.

Results showed that there is an increase in tetracycline residue in poultry liver as a function of time when compared with thigh and chest. This means that the metabolism process in the liver was affected starting from the second day of the experiment.

The average of the percentage ratio of tetracycline residues in the liver increased from 1.240, 1.615, 2.691% on the first day, to 4.061, 5.339, 8.935% on the fourth day of the experiment.

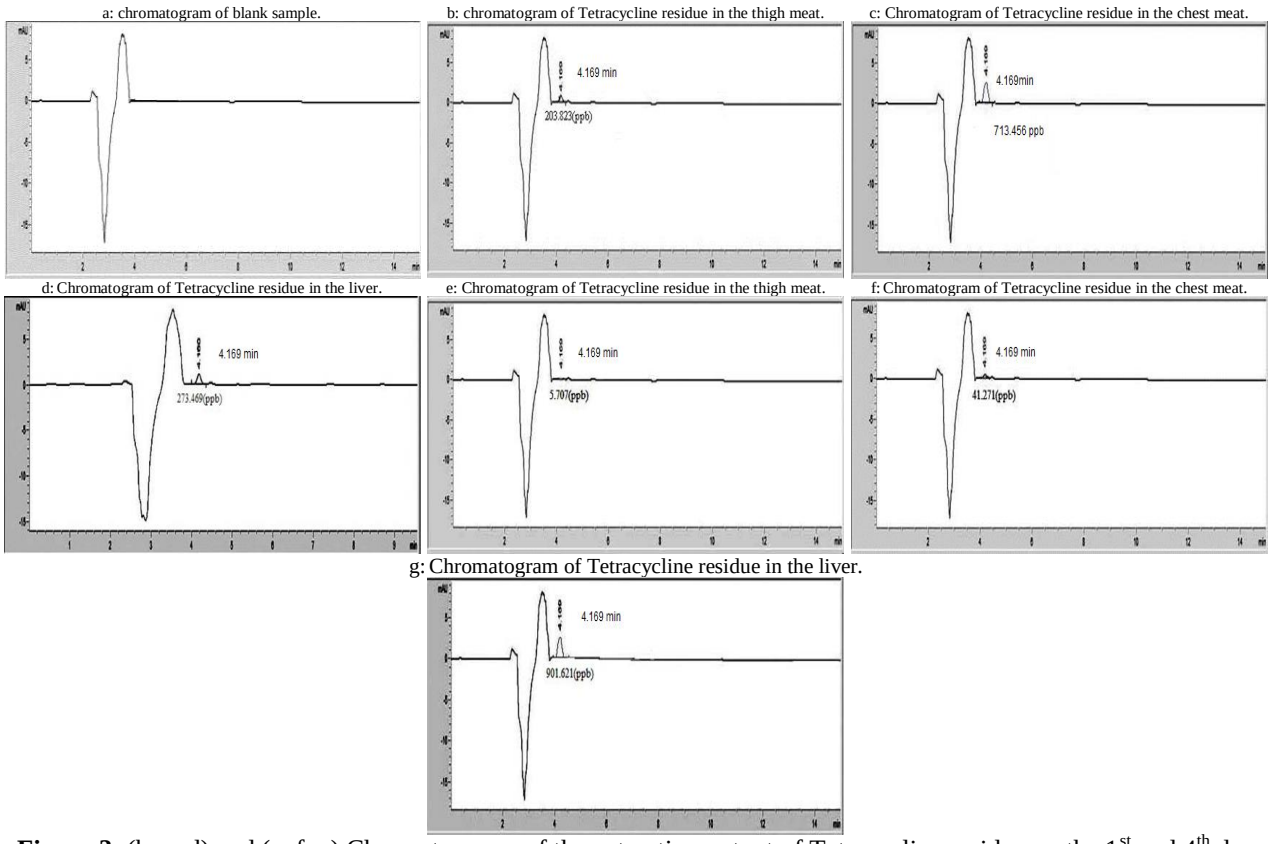

Figure 3: (b, c, d) and (e, f, g) Chromatograms of the extracting output of Tetracycline residue on the 1st and 4th day respectively of the study for one poultry meat sample (thigh, chest, liver). The injected volume in the 1st day was $10.0x10^3$ ppb**.**

Figures 3 showed some chromatograms of tetracycline residues in the thigh, chest and liver of a poultry during the study course.

The chromatograms showed a main peak of tetracycline, and two small impurity peaks, which may possibly be off-scale extraction and purification method. However, these small peaks did not affect the intensity of the tetracycline peak.

On the other hand, other studies showed that tetracycline values were significantly higher in liver and kidney than intestine [13][14]. Drug excretion via egg was 3-fold higher for TC than for CTC, The drug was excreted preferentially into the yolk (about 75% of the total amount) and the elimination period lasted between 6 and 11 days for TC and 9 days for CTC [15]. After words, treatment of hens with tetracycline should follow the proposals presented by the joint FAO/WHO Expert Committee on Food Additives.

Validation Method

Criteria of validation for the analytical method used to determine the tetracycline residue in the chest, thigh and liver samples were summarized as follows:

***1. LOD and LOQ*:** Standard deviation of background noise values of HPLC-DAD signal before the separation process of tetracycline residue was one of the employed methods for calculation of the detection limit LOD. This method need a stabile DAD detector.

Table 6 illustrated the average rise in the background noise of DAD signal (0.0034 mAU.min) according to standard deviation level of 0.000966. LOD and LOQ, which were calculated using the following two relations [16][17]:

LOD=3×SD/gA (µg/kg)=3×0.000966/0.00643 (µg/kg)=0.451 µg/kg(ppb).

LOQ=10×SD/gA(µg/kg)=10×0.000966/0.00643 (µg/kg)=1.502 µg/kg(ppb).

SD: standard deviation of average rise in signal background noise value. gA: the slope of the calibration curve of tetracycline in Figure 2.

Table 6: the values of the signal background noise due to DAD detector.

n (Number of noise signal peaks)	1	2	3	4	5
The values of baseline noise height	0.004	0.003	0.005	0.002	0.003
n (Number of noise signal peaks)	6	7	8	9	10
The values of baseline noise height	0.003	0.004	0.004	0.002	0.002
$\bar{n}\pm$ SD =0.0034 ±0.000966					

Table 7 illustrated a comparison between both values of LOD and LOQ of this study and according to referential studies.

Table 7: Comparison between LOD and LOQ values according to this study and some referential studies.

Reference	[17]	[18]	[19]	[20]	This study
LOD (µg/kg)	7	5	2.5	1.5	0.451
LOQ (µg/kg)	25	13	-	-	1.502

The value of the LOD and LOQ of this study are considered excellent when compared with reference values (5 and 13 µg/kg) [18] and (7 and 25 µg/kg) [17].

Table 8: Repeatability of recovery values for tetracycline 200ppb from poultry chest, thigh and 600ppb from liver samples.

Chest (200 ppb)			Thigh (200 ppb)			Liver (600ppb)		
Conc.*	**Rec. %****		**Conc.***	**Rec. %****		**Conc.***	**Rec. %****	
179.617	89.809	$\overline{Conc.}^{***} = 180.157$ $SD^{****} = 1.187$ $\overline{Rec.}\pm RSD\ \% = 90.079 \pm 0.659\%$	174.851	87.426	$\overline{Conc.}^{***} = 171.612$ $SD^{****} = 1.685$ $\overline{Rec.}\pm RSD\ \% = 85.806 \pm 0.982\%$	499.958	83.326	$\overline{Conc.}^{***} = 499.067$ $SD^{****} = 1.970$ $\overline{Rec.}\pm RSD\ \% = 83.178 \pm 0.395\%$
181.620	90.810		174.620	87.310		499.218	83.203	
182.109	91.055		171.649	85.825		498.200	83.033	
180.333	90.167		175.333	87.667		499.440	83.240	
178.910	89.455		171.906	85.953		497.275	82.879	
177.931	88.966		169.491	84.746		500.598	83.433	
178.998	89.499		170.922	85.461		502.125	83.688	
181.414	90.707		170.416	85.208		500.523	83.421	
181.151	90.576		171.321	85.661		497.428	82.905	
181.776	90.888		170.186	85.093		493.186	82.198	
181.489	90.745		172.476	86.238		498.556	83.093	
178.032	89.016		170.932	85.466		497.359	82.893	
179.906	89.953		169.246	84.623		500.953	83.492	
179.400	89.700		170.290	85.145		498.737	83.123	
180.443	90.222		172.673	86.337		499.850	83.308	
180.000	90.000		171.670	85.835		498.857	83.143	
179.954	89.977		170.979	85.490		498.086	83.014	
180.030	90.015		171.030	85.515		500.019	83.337	
180.402	90.201		170.402	85.201		499.009	83.168	
179.617	89.809		171.851	85.926		501.958	83.660	

n = 4: Number of replicate times of each extract. *: The average of Tetracycline residues for four replicates of one independent sample (ppb). **: The average of the recovery percentage of Tetracycline for four injections of one independent sample. $\overline{Conc.}^{***}$ = the average concentration of Tetracycline residue for 20 replicates. SD ****: The standard deviation for repeatability values of Tetracycline extracted from 20 poultry chest, thigh and liver samples.

***2. Recovery*:** Table 8 showed reduced recovery percentages of tetracycline extracted from 20 samples of poultry chest, thigh and liver and ranged between (88.966 - 91.055%), (84.623 - 87.667%) and (88.966 - 91.055%), respectively with RSD%<1%.

Conclusions and Recommendations

1. Table (3) showed an average decreased of the percentage ratio of tetracycline residues in the poultry thigh injected by 1 mL of standard solution $10.0x10^3$ppb. The percentage ratio decreased from 2.010% on the first day to 0.056% on the fourth day of slaughter.
2. Table (4) showed an average decreased of the percentage ratio of tetracycline residues in the poultry chest injected by 1 mL of standard solution $10.0x10^3$ppb. The percentage ratio decreased from 7.114% on the first day to 0.376% on day four of slaughter.
3. Tables (3, 4) showed an increase in tetracycline residues in poultry chest samples compared to those in the thigh samples under the same field experimental conditions. This was due to the mobile permanent of thigh member compared with the chest of poultry.
4. Table (5) showed an increased average of the percentage ratio of tetracycline residues in the poultry liver injected by 1 m*l* of standard solution $10.0x10^3$ppb. The percentage ratio increased from 2.671% on the first day to 8.898% on day four of slaughter. This may due to over accumulation of tetracycline in the liver over time.
5. Tables (3) showed increased level of tetracycline residues if compared with the injected standard solution $10.0x10^3$ ppb, on the first day of slaughter, with a value of MRL = 200ppb in the thigh. In addition, increased level of tetracycline residues was observed in the chest on the first two days of slaughter. Table (5) showed increased level of tetracycline residues if compared with the injected standard solution $10.0x10^3$ppb in the third and fourth days of the slaughter, with a value of MRL = 600ppb in the liver
6. The value of the LOD = 0.451ppb, LOQ = 1.502 ppb in this study were considered excellent.
7. The range of the average of percentages recovery ratios of tetracycline 200ppb for 20 samples of poultry chest, thigh, and liver shown in (Table 8) were (88.966 - 91.055%), (84.623 - 87.667%) and (82.198 - 83.688 %) respectively.
8. An easy and quick method was developed to determine tetracycline residues in poultry tissues with a good separation and high sensitivity. This method permitted to analyze various tissue samples such as high, chest, liver.
9. We are suggested here an efficient method for determination of tetracycline residue in poultry, using HPLC-DAD, with good recoveries.

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