Validation of the analytical method for determination of tetracycline residues in poultry chest, thigh and liver by HPLC-DAD technique

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Abstract. An easy and quite quick method was developed to determine tetracycline residues in poultry tissues with a good separation and a high sensitivity. This method permitted to analyze various tissues (thigh, chest, liver). Validation of the analytical method is investigated to check if the method’s analytical objective is accomplished, which is obtaining analytical results with an appropriate degree of uncertainty or a good level of confidence. The validation measures of the analytical method were recorded as follows: Limit of Detection (LOD) 0.451 ppb, Limit of Quantification (LOQ) 1.502 ppb, linear correlation coefficient 0.999066 within a range of concentrations between (100.0 - 300.0 ppb), Decision Limit CCα 201.946, 202.763, 603.231 ppb, Detection Capability CCβ 203.892, 205.527, 606.462 ppb for Poultry chest, thigh and liver, respectively, recovery percentages of Tetracycline at a concentration of 200.0 ppb for 20 sample Rec.% (88.966 - 91.055%), (84.623 - 87.667%), (82.198 - 83.688%) for Poultry chest, thigh and liver respectively with a percentage relative standard deviations (RSD%) of < 1 %.

1. Introduction

Many antibiotics are commonly used for preventing and treating several diseases, as well as for promoting growth in food-producing animals and feed efficiency [1, 2]. The intensive production of food from animals has led to the extensive use of antibiotics for growth-promoting pur-poses, treatment of disease and prophylaxis [3, 4]. In veterinary medicine, tetracyclines are used routinely for prevention, control, and reducing cases of disease amongst animals [2, 5]. Tetracycline residues have harmful effects on human health, the soil and water ecosystem, through a series of physical, chemical, and biological processes [6]. In addition, clinical and in vitro observations raised antibiotic resistance could be attributed to the presence of TCs residues in edible and non-edible tissues of intensive animal farming intended for animal and human consumption [7].

The Codex Alimentarius Commission of the FAO/WHO has set for the residues, maximum residue limits MRLs of tetracycline are 200 ppb in muscle, and 600 ppb in liver [8]. Multi-residue methods, which will 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. The use of diode array detector (DAD) as a high performance liquid chromatographic (HPLC) detector has proven to be a powerful tool in the determination and identification of compounds as it makes possible the on-line acquisition of their UV spectra. Furthermore, most of the methods listed
Above are only for one class of antibiotics [9]. Fig. 1 indicates the chemical structure of the tetracycline hydrochloride.

Aim and importance of Research: Validation of the analytical method is investigated to check if the analytical aim of the method is accomplished, which is obtaining analytical results with an acceptable uncertainty level or a good confidence level [8]. The importance of this research include validation of the analytical method followed by identifying each of LOD, LOQ, Linearity, CCα, CCβ, repeatability, recovery, precision and accuracy.

### Figure 1. Chemical structure of the tetracycline hydrochloride

**Definition of validation parameters:**

1. LOD is the lowest quantity of an analyte in a sample which can be detected but not generally quantified as an exact value at signal to noise ratio of 3 [10].
2. LOQ is the smallest quantity to be analyzed at a signal-to-noise ratio of 10 with reasonable precision and accuracy [10].
3. Linearity: The calibration curve is the relationship between the instrument response and concentration of known analytes [11]. The working range of the curve should be defined, the mathematical formula of the curve should be described, the goodness-of-fit of the data to the curve and the acceptability ranges for the parameters of the curve should be described when calibration curves are used for quantification of at least five levels (including zero) in the curve construction. When serial calibration based on a standard solution is required, acceptable ranges shall be indicated for the calibration curve parameters that can differ from series to series [12].
4. CCα is the sample's limit is considered to be truly violate (more than the MRL) with an error likelihood of α = 5% [10].
5. CCβ is the smallest material of analyte that can be detected, classified and quantified in an error likelihood sample that should be less than or equivalent to 5% [10].
6. The repeatability of an analytical method means that the method has the ability to yield the same results in repeated analysis of the same sample, performed by the same operator under the working conditions of the equipment. The procedure was repeated on 4 different days in order to determine inter-day precision [13].
7. Recovery (%): The method accuracy was assessed by recovery test. The recovery of an analytical method is defined as the parameter measuring the efficiency that method has in the analytes extraction process [11].
8. Precision: Precision is a measure of results variability, usually defined by relative standard deviation (RSD %) of a set of replicate results and generally relates to the within-laboratory error (repeatability) of a method [11].
9. Accuracy is a measurement of systematic deviation of the results obtained from the true value and generally expressed in terms of recovery percentage. The smaller the systematic part of the experimental error, the more accurate is the procedure [13].

## 2. Material and methods

### 2.1. Materials and reagents:
Tetracycline hydrochloride (92.6%), methanol, MeOH (99.8%), acetonitrile, ACN (99.8%), phosphoric acid, H3PO4 (99.5%), disodium hydrogen phosphate anhydrate Na2HPO4 (98%), were obtained from Merck, Darmstadt, Germany. Oxalic acid, H2C2O4.2H2O (98%) was obtained from Qualikems-India. Citric acid, C6H8O7.H2O (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.

2.2. Apparatus and Tools
Filter paper obtained from Zelpa, Belgium. Oasis HLB SPE cartridges (500 mg, 5 ml). Chromatographic Column Agilent ZORBAX Eclipse XDB C8 (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. Liquid chromatography solvents and extracted samples were filtered with Teflon and Nylon filter 0.45 µm obtained from Albet, Germany. The chromatographic system was from Agilent Technologies, Palo Alto, CA, USA, with a diode array detector.

2.3. Preparation of Solutions
Mobile phase: A ternary mixture of oxalic acid (0.01M) and acetonitrile and methanol was prepared according to the following (25:15:60, v/v/v).
EDTA-McIlvaine buffer solution (pH* 2.6), a solution was freshly prepared before use daily by dissolving 11.80 g of citric acid monohydrate C6H8O7 .H2O, and 13.72 g of anhydrous Na2HPO4 and 33.62 g of Na2-EDTA in one liter of de-distilled water. The mixture is preserved in a dark-color bottle in a freezer at a temperature of (-20 °C) until its use [14].
A series of standard solutions were prepared for the Tetracycline in concentrations between 1.0-10 x103 ppb in order to determine the trace residue of the antibiotic using HPLC-DAD.

2.4. HPLC–DAD equipment and conditions:
Chromatographic column C8, mobile phase for HPLC was prepared by mixing the solution methanol: acetonitrile: oxalic acid solution (0.01M) (25:15:60, v/v/v), respectively. 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 Rt: 4.169 min. Table 1 Illustrate the analytical conditions of HPLC-DAD analysis of tetracycline [15]. Table 1 Illustrate the analytical conditions of HPLC-DAD analysis of tetracycline.

| Mobile Phase | Acetonitrile: methanol: oxalic acid (0.01M) (25:15:60, v/v/v) |
|--------------|---------------------------------------------------------------|
| Column temperature | 40 °C |
| Wavelengths | λ max = 269 nm |
| Flow Rate | 1 ml/min |
| Retention Time | R t = 4.169 min |
| Chromatography Column | C8 (250x4.6 mm, id., 5 µm) |
| Injection Volume | 20 µl |

2.5. Sampling and preparation
32 poultry birds were selected of 21 days age, which were placed for nine days at an antibiotic-free nutrition system. Birds were fed by milled corn grains only prior to reaching the age suitable for the study. The birds were slaughtered and all skin and fat layers were removed and samples collected from the chest, thigh, and liver of each one bird.
The studied samples were prepared according to the following extraction conditions [16]:

- A 2 g of chest, thigh or liver is weighed.
- The sample is milled and homogenized using a laboratory milling machine. During milling 2 ml of the solvent used for extraction (McIlvain–EDTA at pH* 2.6) was added, then homogenized with 30 ml of the same solvent for only one minute. The sample mixture is processed by an ultrasonic wave at a temperature of 30 °C for half an hour, and then the resulted sample is centrifuged for 15 minutes.
- At the end of the centrifugation process, the liquid layer is separated and filtered using filtration paper.
- The extracted (protein–free) liquid layer is passed onto solid phase extraction cartridge (SPE-Oasis HLB) in order to isolate the antibiotic.
- Tetracycline is eluted from the extraction cartridge with 3.5 ml of methanol. The final extract is passed through a 0.45 μm size filter which becomes ready for injection into (HPLC–DAD).

2.6. 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:

\[
\text{residue percentage \%} = \frac{\text{Residue average concentration (ppb)}}{\text{Injected solution concentration (ppb)}} \times 100 \quad (1)
\]

2.7. Preparation of standard solutions

The standard stock solution: Exactly 10.820 mg of tetracycline hydrochloride was dissolved in a suitable volume of methanol in a 10.0 ml standard flask. The final dissolution process was completed in an ultrasonic water bath for five minutes, and then the solution was diluted to the graded mark with methanol, to obtain a standard solution of concentration 1.0x10^6 ppb of tetracycline. The data was labeled before the flask being covered with an aluminum foil and stored in a freezer 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 x 10^3 ppb was obtained. The task was provided with data label and packed with an aluminum foil and stored in a freezer at -20 °C to be used in preparing the 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, 10000.0 ppb were prepared.

2.8. 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 trace Tetracycline residues in poultry chest, thigh and liver samples according to the analytical conditions stated in Table 1. The corresponding calibration curve based on the relationship between peak area and concentration as illustrated in figure 2.
Figure 2. Three calibration curves of Tetracycline a) Concentration range 1.0- 100.0 ppb 
b) Concentration range 50.0- 2000.0 ppb, c) Concentration range 1000.0-10000.0 ppb.

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  |
Table 2 and figure 2 show excellent linearity 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 1.0-100.0 ppb, 50.0-2000.0 ppb, 1000.0-10000.0 ppb respectively.

3. Results

Criteria of validation for the analytical method applied in determining the Tetracycline residue in the chest, thigh and liver samples were summarized as follows:

3.1 LOD and LOQ

Standard deviation of background noise values of HPLC-DAD signal after the stability of DAD detector before the separation process of Tetracycline residue is one of the employed methods for calculation of the detection limit LOD.

Table 3 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 were calculated using the following two relations [1]:

$$LOD = 3 \times SD / gA \text{ (ppb)} = 3 \times 0.000966 / 0.00643 = 0.451 \text{ (ppb)}.$$  

$$LOQ = 10 \times SD / gA \text{ (ppb)} = 10 \times 0.000966 / 0.00643 = 1.502 \text{ (ppb)}.$$  

3.2. Standard deviation $SD$

Standard deviation of average rise in signal background noise value. $gA$: the slope of the calibration curve of Tetracycline in figure 2.

| $n$ (Number of noise signal peaks) | 1   | 2   | 3   | 4   | 5   | 6   | 7   | 8   | 9   | 10  |
|-----------------------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| The values of baseline noise height | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| $n \pm SD = 0.0034 \pm 0.000966$ |

Table 4 illustrates a comparison between both values of LOD and LOQ according to this study and to referential studies.

Table 4. Comparison between LOD and LOQ values according to this study and some referential studies.

| Reference | This study |
|-----------|------------|
| LOD (ppb) | 0.451      |
| LOQ (ppb) | 1.502      |
The value of the LOD and LOQ according to this study are considered excellent in comparison with the values mentioned in the paper [1] (5 and 13 ppb respectively), and in [17] (7 and 25 ppb respectively).

3.3. Linearity

Single-point calibration (external standard) is designed to meet the specifications are a linear response function and the zero intercept [20]. The calibration curve for tetracycline according to the method of standard additions using a series of standard solutions between 10.0 – 300.0 ppb Table 5 was established as in Figure 3, then 1 ml of each concentration was added to samples of blank poultry chest free of any type of antibiotics [21]. Following this, the extraction conditions were applied in sample preparation and optimum analysis conditions as shown in Table 1 in order to determine rates of tetracycline in the studied samples.

A quantity of 20 µl of each extracted sample was injected four times, then we drafted the standard curve based on the function of peak area of tetracycline (y) by meaning of concentration (x), Figure 3. Excellent linearity was noted with correlation coefficient of \( R^2 = 0.999066 \) resulted from four extraction repetitions of Tetracycline according to the method of standard additions within a range of 10.0 – 300.0 ppb. If the correlation coefficient was at least 0.990, the linearity was deemed acceptable [22].

| Table 5. Peak area averages of tetracycline residues according to the standard additions. |
|--------------------------------------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|
| Con. (ppb)                          | 10.0      | 50.0      | 100.0     | 150.0     | 200.0     | 250.0     | 300.0     |
| Area (mAu.min)                      | 0.491     | 2.634     | 5.264     | 7.984     | 10.040    | 13.225    | 15.568    |

n=4: Peak area averages of tetracycline for four replicates of each level.

![Calibration curve of extracted tetracycline 10.0-300.0ppb](image)

Figure 3. Calibration curve of extracted tetracycline at 10.0-300.0ppb from poultry chest.

Linearity was evaluated by the use of matrix matched calibration curves for each compound. Matrix matched calibration curves were constructed using analyte peak area versus concentration analyte. The coefficient of determination (R2) for the six-point calibration curves above 0.98 [23].

3.4. CCα & CCβ values

CCα & CCβ were determined based on MRL concentrations of (200 ppb) by adding 1ml of tetracycline standard solution based on MRL concentration of 200 ppb to each sample of poultry chest, thigh and liver free of any type of antibiotics under a number of 20 samples [24]. Extracted conditions and optimal analytical conditions were applied in order to determine the recovery percentage of Tetracycline in each sample using the calibration curve illustrated in fig. 2.
CCα and CCβ are calculated as the following [25]:

\[
CCα = MRL + 1.64SD; \quad \alpha = 1.64SD, \quad CCβ = CCα + 1.64SD; \quad \beta = CCβ - MRL
\]

SD: The standard deviation of recovery values of tetracycline residues extracted from 20 samples of poultry thigh, chest and liver as shown in (Table 6).

**Table 6.** Repeatability of recovery values for tetracycline 200 ppb from poultry chest and thigh and 600 ppb from liver samples.

| Sample Type | Conc.* | Conc. | %± Rec. | Conc.* | Conc. | %± Rec. | Conc.* | Conc. | %± Rec. |
|-------------|--------|-------|---------|--------|-------|---------|--------|-------|---------|
| Chest (200 ppb) | 179.617 | 179.767 | 0.157 | 180.402 | 180.461 | 0.059 | 180.030 | 180.099 | 0.070 |
| | | | | | | | | | |
| | 179.954 | 179.979 | 0.025 | 180.000 | 180.070 | 0.070 | 180.333 | 180.414 | 0.080 |
| | | | | | | | | | |
| Thigh (200 ppb) | 171.851 | 172.673 | 0.822 | 170.402 | 170.416 | 0.014 | 171.030 | 170.922 | 0.070 |
| | | | | | | | | | |
| | 170.979 | 170.998 | 0.019 | 171.086 | 171.151 | 0.065 | 170.976 | 171.141 | 0.065 |
| | | | | | | | | | |
| Liver (600 ppb) | 181.489 | 181.776 | 0.287 | 181.151 | 181.414 | 0.263 | 181.414 | 181.455 | 0.041 |
| | | | | | | | | | |
| | 181.776 | 181.897 | 0.123 | 179.617 | 179.617 | 0.000 | 182.109 | 182.109 | 0.000 |

Conc. = 180.157 ppb, SD **** =1.187. Rec. ± RSD % = 90.079±0.659%, CCα = 201.946, CCβ = 203.892.

Conc. = 171.612 ppb, SD **** =1.685. Rec. ± RSD % = 85.806±0.982%. CCα = 202.763, CCβ =205.527.

Conc. = 499.067 ppb, SD **** =1.970. Rec. ± RSD % =83.178±0.395%. CCα = 603.231, CCβ = 606.462.

**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. ***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.

It can be noticed that the results of α tetracycline compound was 1.946, 2.763, 3.231 which is less than 5% in samples of poultry chest, thigh and liver respectively, which means that the determination of tetracycline is statistically acceptable, and the β 3.892, 5.527, 6.462 would fall around 5%, which means that tetracycline compound can be determined with a 95% confidence level for the chest and thigh, but with a confidence level approximately less than 95% for the liver [10].

Table 6 shows reduced recovery percentages of tetracycline extracted from 20 samples of poultry chest, thigh and liver, and ranged 88.966 - 91.055% - 84.623 - 87.667% and 88.966 - 91.055%, respectively with RSD%<1%.

**3.5. Repeatability and correspondence**
Repeatability and correspondence were achieved after tetracycline extraction and separation with concentrations of 100.0, 150.0, 200.0 ppb added to poultry chest, thigh and liver free of any type of antibiotics. The procedure was achieved by the same analyser and in the same laboratory over a period of two weeks based on the extraction conditions for the sample and optimum analytical conditions of HPLC-DAD as shown in Table 1.

Figure 4 and Table 7 illustrate good repeatability values of tetracycline peaks area within the range of the studied concentrations over a period of four consequential days and then after ten days according to excellent correlation coefficients of relative standard deviation of less than 5 % within the study period.

Table 7. Peaks area of tetracycline at 1.0- 200.0 ppb.

| Day | Con.(ppb) | 1.0 | 5.0 | 10.0 | 50.0 | 100.0 | 150.0 | 200.0 | R² *** | Y = a X + b**** |
|-----|-----------|-----|-----|------|------|-------|-------|-------|--------|----------------|
| 1   | peak Area* | 0.062| 0.324| 0.608| 3.211| 6.424 | 9.443 | 12.494| 0.999830| Y = 0.063X + 0.024 |
|     | RSD%***    | 2.840| 2.901| 3.817| 1.041| 1.482 | 1.731 | 1.082 |        |                |
| 2   | peak Area* | 0.060| 0.328| 0.611| 3.205| 6.423 | 9.440 | 12.495| 0.999839| Y = 0.063X + 0.023 |
|     | RSD%***    | 2.829| 0.294| 0.70  | 1.109| 0.116 | 1.862 | 0.50  |        |                |
| 3   | peak Area* | 0.061| 0.323| 0.606| 3.210| 6.419 | 9.438 | 12.492| 0.999836| Y = 0.063X + 0.023 |
|     | RSD%***    | 3.282| 0.738| 5.442| 1.197| 0.307 | 0.316 | 1.629 |        |                |
| 4   | peak Area* | 0.059| 0.322| 0.608| 3.209| 6.40  | 9.421 | 12.399| 0.999766| Y = 0.063X + 0.029 |
|     | RSD%***    | 2.215| 0.794| 1.674| 0.311| 0.129 | 0.604 | 1.717 |        |                |
| 5   | peak Area* | 0.061| 0.315| 0.674| 3.222| 6.418 | 9.646 | 12.597| 0.999847| Y = 0.065X + 0.009 |
|     | RSD%***    | 4.726| 2.70 | 1.515| 0.541| 0.113 | 0.931 | 0.994 |        |                |

*: Peak area averages of standard tetracycline for four replicates of injection for each concentration. **: The average of relative standard deviation for each concentration. R² ***: correlation coefficient of the calibration curve of tetracycline per day. ****: Calibration curve equation.

Table 8 shows a recovery percentage to study the extraction and separation of tetracycline ranged between (88.49-90.87%), (88.89-93.93%) and (87.74-95.21%) of poultry chest, and (85.10- 88.35%), (84.43-89.93%) and (86.61-89.77%) of poultry thigh, and (80.09-81.77%), (80.13-84.54%) and (80.40-84.75%) of poultry liver for the three concentrations (100, 150, 200 ppb) respectively. This was associated with a percentage relative standard deviation less than 2.6%. This study gives a clear image for the accuracy of the adopted analytical method and extraction procedures.
Figure 4 Calibration curves of tetracycline compound resulting from the repeatability analytical method approved study.

Table 8. Repeatability extraction of tetracycline within two weeks

| Day | 1  | 2   | 3   | 4   | 15  |
|-----|----|-----|-----|-----|-----|
|     | Con.(ppb) | 100.0 | 150.0 | 200.0 | 100.0 | 150.0 | 200.0 | 100.0 | 150.0 | 200.0 | 100.0 | 150.0 | 200.0 |
|     | Con.(ppb)' | 89.29 | 140.90 | 188.40 | 90.48 | 135.10 | 187.69 | 89.59 | 133.33 | 190.41 | 88.49 | 137.46 | 174.74 | 90.87 | 133.69 | 179.55 |

Calibration curve of tetracycline 1.0-200.0ppb for second day

Calibration curve of tetracycline 1.0-200.0ppb for third day

Calibration curve of tetracycline 1.0-200.0ppb for fourth day

Calibration curve of tetracycline 1.0-200.0ppb for fifteenth day
3.6 Accuracy and precision

Precision was measured as an average of the percentage relative standard deviation for a set of repeat measurement results for the recovery of the added standard solution to the sample at different concentrations (100.0, 150.0, 200.0ppb). The accuracy of the analytical procedure is expressed as relative error of measurement, and can be calculated by the equation (2) [26]:

$$RE\% = \frac{\text{Mean measured values} - \text{added amount}}{\text{added amount}} \times 100 \tag{2}$$

Table 8 shows the average of the percentage relative standard deviation of tetracycline concentrations less than 2% per day or between the successive days. It gives a clear image for the precision of the analytical method and extraction procedures adopted in this study. The accuracy values of the analytical method used ranged between -4.795 and -19.910.

In many countries, tetracycline antibiotics (TCs) are widely used for the treatment of poultry. Certain aspects of usage of TC, such as standard applications, dosages, etc., have been thoroughly reviewed and studied. However some difficulties remain in trying to compare the pharmacokinetics (PK) of TCs, which constitute an important gap in our knowledge. Such data helps us to understand how long a drug would be present in an organism, which has obvious repercussions for the product est, and (80.13 - 85.10) for thigh, and (85.10 - 126.80) for liver shown in (Table 6) were (88.966 - 91.055%), (84.623 - 87.667%) and (82.198 - 83.688%) respectively. The range of the average of percentages recovery ratios of tetracycline 200 ppb for 20 samples of poultry chest, thigh and liver respectively, with percentage relative standard deviation RSD% < 1%. The range of the average of percentages recovery ratios of tetracycline were (88.49 - 90.87%), (88.89 - 93.93%) and (87.37 - 95.21%) for chest, and (85.10 - 88.35%), (84.43 - 89.93%) and (86.15 - 89.77%) for thigh, and (80.09 - 81.77%), (80.13 - 84.53%) and (80.40-84.75%) for liver at concentrations of (100, 150, 200 ppb) respectively, with a percentage relative standard

| Concentration (ppb) | Percentage Recovery (Rec.%) | RSD% | RE% |
|---------------------|-----------------------------|------|-----|
| 100                 | 89.20                        | 0.01 | -10.8 |
| 150                 | 93.93                        | 0.02 | -6.02 |
| 200                 | 94.20                        | 0.02 | -5.80 |
| 400                 | 90.48                        | 0.03 | -9.52 |
| 500                 | 90.07                        | 0.04 | -9.91 |
| 1000                | 93.85                        | 0.05 | -6.16 |
| 2000                | 89.59                        | 0.06 | -10.21 |
| 5000                | 88.89                        | 0.07 | -9.21 |
| 10000               | 86.49                        | 0.08 | -9.86 |
| 20000               | 85.21                        | 0.09 | -10.01 |
| 50000               | 89.49                        | 0.10 | -9.51 |

Table 8: Results of accuracy and precision for the recovery of the added standard solution to the sample at different concentrations.

*: The average concentration of tetracycline for four replicates. **: Recovery average of tetracycline for four extracted replicates. ***: The average of the percentage relative standard deviation for each concentration. ****: The average of the percentage relative standard deviation and the percentage recovery for concentrations at the same day. RE%: Percentage relative error.
deviation RSD% < 3%. It gives a clear image for the accuracy of the analytical method and extraction procedures adopted in this study.

An easy and quite quick method was developed to determine Tetracycline residues in poultry tissues with a good separation and a high sensitivity. This method permitted to analyze various tissues (thigh, chest, liver).

In this paper, an efficient method for simultaneous determination of Tetracycline residue in poultry, using HPLC-DAD with good recoveries was obtained.

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