CHAPTER 1

Detection of Antibiotic Residues in Aquaculture Products

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Aquacultured animals are under constant threat from bio-aggressors such as viruses, bacteria, parasites and fungi. These organisms harm either spontaneously or through aquatic animal husbandry practices, and often both. Indeed, it is generally recognized that disease problems follow the development of techniques for animal production.

Consequently, fish culture uses a variety of chemicals that represent potential threats to the health of the cultured animal, indigenous biota, and even humans. Chemicals employed in aquaculture include the following:
- Drugs used to treat disease (chemical therapeutants)
- Chemicals introduced through construction materials
- Hormones used to alter reproductive viability, sex, and growth rates

Of these, chemotherapeutic drugs are the most harmful. Chemotherapeutic treatments are initiated after clinical signs of a disease appear in a population of fish. Chemicals used in construction and hormones are not considered because they are relatively non-toxic.

The use of chemical therapeutants obviously leads to the transit of drugs and to their persistence in products intended for human consumption. It also leads to the release of drugs or their metabolites to the aquatic environment. Hence the criticisms raised in the press against the use of chemotherapy in aquaculture, and the restrictive legislation set up in many countries under pressure of public opinion. It sometimes appears that people would believe that drug resistance of bacteria responsible for human infections originates exclusively, or almost exclusively, from consumption of animal products such as those provided by aquaculture.

It should be noted that in addition to the chemicals that are deliberately used, fish raised in aquaculture are also susceptible to contamination via pesticides present in feed, agriculture run-off water, and sediments. The magnitude of human exposure to these sources has not yet been fully assessed and should be examined periodically in light of the growth and change in this sector of the seafood industry.
Determination of Oxolinic Acid by HPLC-Fluorescence Method

Principle

Oxolinic acid (OXA) is a powerful synthetic antibacterial agent used in aquaculture in curing or preventing diseases caused by certain species of Yersinia, Aeromonas and Vibrio.

The methods for the detection of the bactericide, oxolinic acid, can be generally divided into two categories, namely biological and physiochemical. The biological method, such as bioassay, lacks sensitivity and specificity. The physiochemical method, based mainly on HPLC with UV or fluorescence detection, is much faster, more specific and more sensitive than the biological method.

Apparatus


b. Chromatographic column: Reverse phase, TSK-GEL ODS-80TM (150 x 4.6mm)
   Operating condition: Flow rate set at 0.5 mL/min.
   Injection volume: 20 ml.
   As a part of the system shut-down at the end of the experiment, HPLC grade water is pumped through the column for a minimum of twenty min followed by a twenty min rinse with methanol:water (7:3) at 0.5mL/min

c. Filter Unit: GL, Chromatodisc 13P, 0.4 mm

d. Glass centrifuge bottle (150 ml) and tubes (15 mL)

e. Separatory flasks (125 mL)

f. Florentine flask (100 mL)

g. Tissue homogenizer

h. Centrifuge
i. Rotary evaporator
j. Ultrasonic water bath

**Reagents**

a. Acetonitrile (HPLC grade)
b. Water (HPLC grade, Diamond-Q)
c. 1-propanol (GR grade)
d. n-hexane (GR grade)
e. Sodium sulfate, anhydrous (GR grade)
f. Methanol (HPLC grade)
g. Sodium chloride (GR grade)
h. 0.1 M citric acid (HPLC grade): Dissolve 21.01 g of monohydrate salt of citric acid in 1L of HPLC grade water.
i. Oxolinic acid (Sigma Chemical Company): All standard solutions are stored below 10° C. Stock solution is stable for at least 3 months, but diluted solutions should be kept no longer than 2 weeks.

**Stock solution (100 ppm):** Weigh accurately 10 mg of oxolinic acid and make up to 100 mL in a volumetric flask using acetonitrile:water (1:1) solution.

**Intermediate solution (10 ppm):** Pipette accurately 5 mL of the 100 ppm stock solution into a 50 mL volumetric flask and make up volume with acetonitrile:water (1:1) solution.

**Working solution (1 ppm):** Pipette accurately 5 mL of the 10 ppm intermediate solution into a 50 mL volumetric flask and make up volume with acetonitrile:water (1:1) solution.
The working solution should not be used after 1 month of refrigerated storage and fresh working solution should be prepared.

j. Mobile Phase: ( Acetonitrile:methanol) - 0.1M citric acid solution (2:2:3), filtered through polyvinylidene fluoride membrane (0.45 mm). The mobile phase should be prepared daily.
Sampling Procedures

1. Place frozen shrimps sample at 5°C overnight. Remove the head and shell.

2. Mince the sample rapidly and thoroughly with chopper. Remove unground material from the blade of chopper and mix thoroughly with ground material and mince thoroughly again.

3. Turn the mince into the shape of a burger and divide into four equal portions.

4. Take the first quadrant of minced meat for testing.
Procedure

1. Accurately weigh 5 g of the minced sample (edible portion of the tiger prawn, not de-veined) into a 150 ml glass centrifuge bottle. Spike test sample at this stage with 2 ml of 1 ppm working solution.

2. Add 25 ml of acetonitrile and 10 g of anhydrous sodium sulfate into the sample and homogenize for 1 min.

3. Centrifuge at room temperature for 5 min at 2,500 rpm.

4. Filter the supernatant through Whatman No.1 filter paper into a 125 ml separatory funnel containing 25 ml acetonitrile - saturated n-hexane.
5. Add another 25 ml of acetonitrile to the homogenate and sonicate for 30 sec. gently mixing the sample with a glass rod during this process.

6. Centrifuge at room temperature for 5 min at 2,500 rpm and filter the supernatant into the earlier 125 ml separatory funnel (Step 4). Rinse the filter paper with acenotrile and allow washing to drain into separatory funnel

7. Shake flask vigorously for 10 min. Allow to stand for separation into layers.

8. Slowly drain off the acetonitrile (lower) layer into a 100 ml florentine flask.
9. Add approximately 5 ml of 1-propanol (add more if mixture boils too vigorously) and evaporate the acetonitrile layer to dryness using the rotary evaporator at 40°C.

10. Flush flask with nitrogen gas to remove any trace of propanol.

11. Add accurately 2 ml of acetonitrile:water (3:7) and sonicate until the residue is dissolved.
12. Transfer the sonicated solution into a 15ml glass centrifuge tube. Add 100 ml of sodium chloride and 1 ml of acetonitrile-saturated n-hexane and sonicate the mixture. At this stage, an orange-layer develops at the top. This is a sign of lipids moving on to the top n-hexane layer.

13. Centrifuge at room temperature for 5 min at 2,500 rpm.

14. Very carefully, using a pasteur pipette, pipette out the acetonitrile (lower, aqueous) layer into a plastic syringe (fixed with filter Chromatodisc 13P) and filter the extract into a glass vial.

15. The filtered sample is then ready for injection into HPLC. Inject 20 µl sample using a flow rate of 0.5 ml/min with detector excitation set at 337 nm and emission at 365 nm.
Calculation

Concentration of oxolinic acid in sample (ppm) =

\[
\text{Std conc. (mg) } \times \frac{\text{Sample peak area}}{\text{Std. peak area}} \times \frac{\text{Final vol. of extract}}{\text{Sample weight}} \times F \times \frac{\text{Inj. vol. (sample)}}{\text{Inj. vol. (std)}}
\]

where \( F = \) dilution factor = 1

Method Validation

Method validation was performed in compliance with regulations to ensure that the analytical methodology is accurate, specific, reproducible, and adaptable over the specified range of an analyte.

System suitability tests are used to verify that the resolution and reproducibility of the system are adequate for the analysis to be performed. The system suitability was checked by injecting 2 ppm of oxolinic acid standard for 10 times into the HPLC. Percentage relative standard deviation calculated from the 10 injections is 1.8 % (criteria: %RSD \(< = 2\%\)). This indicates that the autosampler of HPLC is suitable for use.

All the criteria like Number of theoretical plates (N), Tailing factor (T), Precision (%RSD) and Resolution (R) were met. The results are shown in Table 1. The retention time of oxolinic acid is about 6.5 min. Analysis time per injection is only 9 min, which is practical. Thus, this shows that flow-rate, column length, and solvent composition are suitable for this application. Relative standard deviation of retention time between replicates is < 1%. T, was determined by calculating the asymmetrical factor derived from generated chromatogram. Calculated T was found to be about 1.6 (criteria: 0.5 \(< = T = < 2\)) and N, was 3306 (general criteria: N \(> = 2000\)). The N and T values indicate that the column used, TSK-GEL ODS-80 TM, is suitable for this application.
Table 1. System suitability of the HPLC-fluorescence detector system for oxolinic acid determination.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Oxolinic acid*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retention Time (min)</td>
<td>6.481± 0.018</td>
</tr>
<tr>
<td></td>
<td>%RSD = 0.28</td>
</tr>
<tr>
<td>Area</td>
<td>4697763 ± 84561</td>
</tr>
<tr>
<td></td>
<td>%RSD = 1.80</td>
</tr>
<tr>
<td>Height</td>
<td>229946 ± 4287</td>
</tr>
<tr>
<td></td>
<td>%RSD = 1.86</td>
</tr>
<tr>
<td>W₁/₂ (min)</td>
<td>0.266 ± 0.008</td>
</tr>
<tr>
<td></td>
<td>%RSD = 2.90</td>
</tr>
<tr>
<td>A (min)</td>
<td>0.317 ± 0.000</td>
</tr>
<tr>
<td></td>
<td>%RSD = 0.00</td>
</tr>
<tr>
<td>B (min)</td>
<td>0.205 ± 0.013</td>
</tr>
<tr>
<td></td>
<td>%RSD = 6.15</td>
</tr>
<tr>
<td>Tailing Factor (min)</td>
<td>1.60 ± 0.10</td>
</tr>
<tr>
<td></td>
<td>%RSD = 6.00</td>
</tr>
<tr>
<td>Resolution (R)</td>
<td>7.41 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>%RSD = 0.28</td>
</tr>
<tr>
<td>Theoretical Plates (N)</td>
<td>3306 ± 203</td>
</tr>
<tr>
<td></td>
<td>%RSD = 6.13</td>
</tr>
</tbody>
</table>

* Mean ± Standard Deviation

Criteria:
1. Number of theoretical plates, \( N \geq 2000 \) \([N=16(tR/W)^2]\)
2. Tailing Factor, \( 0.5 \leq T \leq 2 \)
3. Precision \( \text{RSD} \leq 2 \% \) in 10 injections of the standard
4. Resolution, \( R \geq 2 \) between adjacent peaks \([R = (tR1-tR2)/0.5(w1+w2)]\)

Calibration curves were generated using 0.2, 0.5, 1.0 and 2.0 ppm of the oxolinic acid standards. A good correlation \((r = 0.999)\) was obtained between concentration and peak area.

Recovery tests were carried out for 7 blanks, 7 spiked blanks, and 7 spiked tiger prawn samples (spiked with 2mL of 1 ppm standard solution). (Table 2). No oxolinic residues were detected for the 7 blank samples. The recoveries for the 7 spiked blanks and 7 spiked tiger prawn samples ranged from 86.50 - 88.60%. The limit of quantification (LOQ) was 0.06 mg/g and the limit of detection (LOD) was 0.02 mg/g. The precision, expressed as percentage relative standard deviation, was below 4.53 %.
Table 2. Percentage recovery of oxolinic acid in spiked blanks and spiked tiger prawn samples.

<table>
<thead>
<tr>
<th>Oxolinic Acid (2 µg per 5 g sample)</th>
<th>% Recovery*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spiked blanks</td>
<td>86.50 ± 7.04</td>
</tr>
<tr>
<td>Spiked samples</td>
<td>88.60 ± 4.01</td>
</tr>
</tbody>
</table>

* Mean ± Standard Deviation

References


Determination of Oxytetracycline, Tetracycline and Chlortetracycline by HPLC - Fluorescence Method

Principle

Tetracycline is widely used in the culture of fish as an antibacterial agent against various fish diseases such as vibriosis, furunculosis, etc., and is generally used along with other drugs or antibiotics. However, high dosage and improper use of antibiotics have resulted in levels of antibiotic residues that are higher than permitted, raising concern over the effects of antibiotics on the environment and on consumer health. The 3 main antibiotics under the tetracycline family that are commonly used as feed additives are oxytetracycline (OTC), tetracycline (TC) and chlortetracycline (CTC). The quantitative method used for the determination of these residues during this study was High Performance Liquid Chromatography (fluorescence detector method).

where
Oxytetracycline : R1 = H, R2 = OH
Tetracycline : R1 = H, R2 = H
Chlortetracycline : R1 = Cl, R2 = H

Apparatus

a. Liquid Chromatography: WATERS Isocratic pump system, WATERS in-line degasser, 600E Multisolvent Delivery System, 600E System Controller, 717 Plus Autosampler equipped with 470 Scanning Fluorescence Detector capable of monitoring emission at 520 nm and excitation at 380 nm.

b. Chromatographic column: Reverse phase, TSK-GEL ODS-80TM (150 x 4.6mm)

Operating condition: Flow rate set at 1 mL/min.
Injection volume: 20 µl
As a part of the system shut-down at the end of the experiment, HPLC grade water is pumped through the column for a minimum of 20 min followed by a 20-min rinse with methanol:water (7:3) at 0.5mL/min.

c. Sep-Pak C18 cartridge (WATERS) with 10 ml plastic syringe
d. Glass centrifuge bottle (150 mL) and tubes (15 mL)
e. Propylene centrifuge bottle (250 mL)
f. Florentine flask (500 mL and 100 mL)
g. Tissue homogenizer
h. Centrifuge
i. Rotary evaporator
j. Ultrasonic water bath

Reagents
a. Oxytetracycline.HCl Standard (Sigma)
b. Tetracycline.HCl Standard (Sigma)
c. Chlortetracycline.HCl Standard (Sigma)
d. Methanol, HPLC grade
e. Magnesium acetate, GR grade
f. Tetrahydrate salt of magnesium acetate, GR grade
g. Disodium salt of Ethylene Diamine Tetraacetic Acid (EDTA), GR grade
h. Monohydrate salt of citric acid
i. Dihydrate salt of disodium hydrogen phosphate, GR grade
j. Petroleum ether, GR grade
k. 5% Disodium salt of Ethylene Diamine Tetraacetic Acid (EDTA): Dissolve 5 g EDTA in 100 mL pure Diamond Q water.
l. Mcllvaine buffer (pH 6.0): Prepare a 0.2 M solution of dihydrate salt of disodium hydrogen phosphate by dissolving 22.71 g of the solute in 800 mL of distilled water. Prepare a 0.1 M solution of monohydrate salt of citric acid by dissolving 10.50 g of the solute in 500 mL of distilled water. To the 500 mL of 0.1 M citric acid solution, slowly add 0.2 M solute of dihydrate salt of disodium hydrogen phosphate and adjust the pH of the buffer to 6.0.
m. 0.1 M EDTA in McIlvaine buffer (pH 5.5): Dissolve 37.224 g of disodium salt of EDTA in 1 L of McIlvaine buffer and check that pH is 5.5.

n. Extraction solution: Mix 0.1 M EDTA in McIlvaine buffer (pH 5.5) and methanol in ratio of 3:7 by volume. It is important to prepare the extraction solution fresh each time before use as white precipitates could form almost immediately, after mixing.

o. 1 M Imidazole buffer (pH 7.2): Dissolve 68.08 g imidazole, 10.72 g magnesium acetate and 0.37 g disodium salt of EDTA in 800 ml of HPLC grade water. Adjust to pH 7.2 using acetic acid and make up to 1 L with HPLC grade water.

p. Mobile Phase solution: Mix 1 M imidazole and HPLC grade methanol in the ratio of 80:20 by volume. It is very important to filter the Mobile Phase solution through a 0.45 µm Millipore filter.

q. Oxytetracycline, Tetracycline and Chlortetracycline (Sigma Chemical Company): All standard solutions are stored below 10°C. Stock solution is stable for at least 3 months, but diluted solutions should be kept no longer than 2 weeks.

Stock solution (1000 ppm): Weigh accurately 100 mg of oxytetracycline, tetracycline, and chlortetracycline standards into three separate 100 mL volumetric flask. Make up to volume with HPLC grade methanol:water (1:1) solution.

Intermediate solution (100 ppm): Pipette accurately 10 mL of the 1000 ppm stock solution into a 100 mL volumetric flask and make up to volume with HPLC grade methanol:water (1:1) solution.

Working solution (5 ppm): To get a mixed standard solution of oxytetracycline, tetracycline and chlortetracycline, pipette 5 mL of each of the three standards (intermediate solutions) to a 100 mL volumetric flask and make up to volume with HPLC grade methanol:water (1:1) solution.

It is best to prepare fresh working solution after one month.
Sampling Procedures

1. Place frozen shrimp sample in 5°C overnight.
2. Remove head and shell.
3. Mince the sample rapidly and thoroughly with a chopper.
4. Remove unground material from blade of chopper and mix thoroughly with ground material and mince thoroughly again.
5. Turn mince into the shape of a burger and divide into equal quarters.
6. Take first quadrant of minced meat for testing.

Procedure

1. Weigh 10 g of the minced sample (edible portion of tiger prawn, not deveined) into a 250 ml plastic centrifuge bottle. (For spiked sample, spike with 2 ml of 5 ppm standard working solution).

2. Add 60 ml of the freshly prepared extraction solution into the centrifuge bottle and homogenize for 1 min.

3. Centrifuge for 5 min at 3,000 rpm.
4. Filter supernatant through Whatman filter paper, No. 41, into a 500 ml florentine flask.

5. Wash residue with another 30 ml of extraction solution and sonicate for 30 sec. Gently mix the sample during this process using a glass rod. Centrifuge for 5 min at 3,000 rpm and filter the supernatant into the same florentine flask.

6. Rinse filter paper twice, with 10 ml of extraction solution each time. Evaporate pooled filtrate to approximately 5 ml, in a water bath set at 40°C.

7. Transfer the concentrated filtrate to a 150 ml centrifuge bottle. Rinse florentine flask with 20 ml of Milli-Q water and pool rinse into the same centrifuge bottle containing the filtrate. Add approximately 20 ml of petroleum ether and shake the mixture for 10 min.
8. Centrifuge for 5 min at 3,000 rpm. Use Komagome pipette to remove most of the petroleum (upper) layer, leaving behind only aqueous layer.

9. To condition the Sep-pak C18 cartridge, attach it to the glass syringe at one end, followed by the following washing steps:
   - Inject the cartridge with 10 ml of methanol and discard the eluate.
   - Elute with 10 ml of Milli-Q water and discard the eluate.

10. Transfer the aqueous layer (from Step 8), into the plastic syringe attached with the conditioned Sep-Pak C18 cartridge (from Step 9). This is the absorption stage of any tetracycline residue to the cartridge.

11. Elute the tetracycline residues with 30 ml of methanol at a flow rate of 1.5 ml/min. Collect the eluate in a 100 ml florentine flask.

12. Evaporate the eluate to complete dryness using the rotary evaporator at 40º C and by flushing with nitrogen gas.
Add 2 ml of mobile phase to the dried residue. Sonicate until clear before injecting into the HPLC.

Calculation

Concentration of tetracycline in sample (ppm):

\[
\text{Std conc. (mg)} \times \frac{\text{Sample peak area}}{\text{Std. peak area}} \times \frac{\text{Final vol. of extract}}{\text{Sample weight}} \times F \times \frac{\text{Inj. vol. (sample)}}{\text{Inj. vol. (std)}}
\]

where \( F = \) dilution factor = 1

Method Validation

The system suitability was checked by injecting a 5 ppm mixed standard (oxytetracycline, tetracycline, and chlortetracycline) for 10 times into the HPLC.

System suitability is the checking of a system to ensure performance before or during the analysis of unknowns. Parameters such as tailing factors, resolution and reproducibility (%RSD retention time and area for replicates) were determined and compared against the specifications set for the method.

All the criteria like Number of theoretical plates (N), Tailing factor (T), Precision (%RSD) and Resolution (R) were met. The results are shown in Table 3. The standard solution at 5 ppm produced 3 distinct peaks within 25 min after injection. This shows that flow-rate, column length and solvent composition is suitable for this application. Resolution between oxytetracycline and tetracycline peaks is 2.0 and at least the same for chlortetracycline with reference to either oxytetracycline or tetracycline (Table 3). The % RSD for retention time ranged from 0.27 to 0.78. The number of theoretical plates and tailing factor indicate that the column used, TSK-GEL ODS-8OTM, is suitable for this application.
Table 3. System suitability of the HPLC-fluorescence detector system for OTC, TC and CTC determination

<table>
<thead>
<tr>
<th>Parameters</th>
<th>OTC*</th>
<th>TC*</th>
<th>CTC*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retention Time (min)</td>
<td>5.063 ± 0.013, %RSD = 0.27</td>
<td>7.777 ± 0.025, %RSD = 0.32</td>
<td>23.950 ± 0.187, %RSD = 0.78</td>
</tr>
<tr>
<td>Area</td>
<td>3014317 ± 56586, %RSD = 1.88</td>
<td>3422706 ± 50176, %RSD = 1.47</td>
<td>1271583 ± 31481, %RSD = 2.48</td>
</tr>
<tr>
<td>Height</td>
<td>143573 ± 15163, %RSD = 1.06</td>
<td>137345 ± 1590, %RSD = 1.16</td>
<td>21901 ± 359, %RSD = 1.64</td>
</tr>
<tr>
<td>W_{1/2} (min)</td>
<td>0.26 ± 0.01, %RSD = 3.70</td>
<td>0.342 ± 0.026, %RSD = 7.55</td>
<td>0.87 ± 0.02, %RSD = 2.34</td>
</tr>
<tr>
<td>A (min)</td>
<td>0.32 ± 0.04, %RSD = 11.58</td>
<td>0.37 ± 0.02, %RSD = 6.15</td>
<td>1.06 ± 0.11, %RSD = 10.00</td>
</tr>
<tr>
<td>B (min)</td>
<td>0.51 ± 0.03, %RSD = 6.04</td>
<td>0.39 ± 0.03, %RSD = 7.53</td>
<td>0.88 ± 0.14, %RSD = 15.58</td>
</tr>
<tr>
<td>Tailing Factor (min)</td>
<td>1.63 ± 0.26, %RSD = 15.87</td>
<td>1.07 ± 0.14, %RSD = 13.39</td>
<td>0.84 ± 0.18, %RSD = 21.15</td>
</tr>
<tr>
<td>Resolution (R)</td>
<td>4.78 ± 0.16, %RSD = 3.45</td>
<td>5.65 ± 0.15, %RSD = 2.68</td>
<td>72.19 ± 3.57, %RSD = 4.95</td>
</tr>
<tr>
<td>Theoretical Plates (N)</td>
<td>2139 ± 168, %RSD = 8.67</td>
<td>2700 ± 234, %RSD = 7.86</td>
<td>3523 ± 340, %RSD = 9.66</td>
</tr>
</tbody>
</table>

* Mean ± Standard deviation

Criteria

1. Number of theoretical plates, N >= 2000 [N=16(tR/W)^2]
2. Tailing Factor, 0.5<=T<=2
3. Precision RSD<= 2 % in 10 injections of the standard
4. Resolution, R >=2 between adjacent peaks [R = (tR1-tR2)/0.5(w1+w2)]

The calibration curves were generated for individual components using 0.5, 2.0, 5.0 and 10.0 ppm of the individual standards. A good correlation (r = 0.999) was obtained between concentration and peak area.
Table 4. Correlation between concentration and peak area

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>Coefficient of correlation, $r$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxytetracycline</td>
<td>0.999</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>0.999</td>
</tr>
<tr>
<td>Chlotetracycline</td>
<td>0.999</td>
</tr>
</tbody>
</table>

Recovery tests were carried out for 7 blanks, 7 spiked blanks, and 7 spiked tiger prawn (spiked with 1 ug/g standard solution). No tetracycline residues were detected for the 7 blank samples. The recoveries for the 7 spiked blanks and spiked tiger prawn were satisfactory. The percentage recoveries for OTC, TC, and CTC were 78.62%, 79.89%, and 68.26% respectively (Table 5).

Table 5. The mean percentage recovery of 7 spiked tiger prawn samples

<table>
<thead>
<tr>
<th>Antibiotics (10 ug per 10 g sample)</th>
<th>% Recovery*</th>
</tr>
</thead>
<tbody>
<tr>
<td>OTC</td>
<td>78.62 ± 1.44</td>
</tr>
<tr>
<td>TC</td>
<td>79.89 ± 0.54</td>
</tr>
<tr>
<td>CTC</td>
<td>68.26 ± 2.14</td>
</tr>
</tbody>
</table>

* Mean ± Standard Deviation

References


Chlortetracycline, Oxytetracycline, and Tetracycline in Edible Animal Tissues, AOAC Official Methods of Analysis 995.09, 2000