Application of a Solid-Phase Fluorescence Immunoassay to Determine Oxytetracycline and Tetracycline Residues in Tissue of Olive Flounder (Paralichthys olivaceus)

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ABSTRACT. ParalluxTM, a solid-phase fluorescence immunoassay (SPFIA) developed for antibiotics residue detection in milk, was applied for analysis of fish tissue. The recommended therapeutic doses of oxytetracycline (OTC, 100 g/ton water, withdrawal period 30 days) and tetracycline (TC, 150 g/ton water, withdrawal period 30 days) were treated to a group of 35 olive flounders (Paralichthys olivaceus) using dipping administration. Muscle was sampled before and after drug treatment 1st, 2nd, 3rd, 5th, 7th, and 14th day. The concentration of oxytetracycline in muscle, determined by SPFIA, was compared with that of internal standard (100 ppb as oxytetracycline). The S/C ratio of sample inhibition value to cutoff inhibition value was employed as an index to determine the muscle residue in olive flounder. To investigate the recovery rate, and standard solutions were added to muscle samples to give final concentrations in muscle of 0.1 and 0.5 µg/ml. The recovery rates of all spiked samples were >89% of the spiked value. OTC and TC were detected in muscle of fishes treated until the 3rd day of withdrawal period. The present study showed that the SPFIA can be easily adopted in predicting tissue residues for OTC and TC in farmed fishes.

KEY WORDS: flounder, solid-phase fluorescence immunoassay, tetracycline antibiotics.

contents were mixed for 1 min in a stomacher. Approximately 2 ml was placed in Eppendorf tubes and centrifuged for 5 min at 20,000 × g. Stock solution of 1 mg/ml of each of OTC and TC were prepared with United States Pharmacopeia (USP) standards in methanol, and stored at −20°C. This standard solution was used for the preparation of both calibration solutions and fortified samples. Just before used, the stock solutions were diluted in muscle extracts from non treated fish, to prepare 0.025, 0.05, 0.1, 0.2, 0.5 and 1.0 µg/ml working standard solutions. To get the recovery rate, the standard solutions were added to muscle samples to give final concentrations in muscle of 0.1 and 0.5 µg/ml. After blending, these samples were extracted as described above then analyzed in a blind fashion.

The sample test with Parallux™ system was performed as prescribed by the manufacturer. After the wells in the reagent tray are filled with 100 µl sample, the contents are mixed with labeled antibodies already present in the wells; thereafter, the mixtures are allowed to react with the solid phase in the capillary tubes. When samples do not contain any analyte reacting with the antibodies, a large amount of labeled antibody remains free to bind to the solid phase. After capillaries are washed and dried, a laser source excites fluorescence, and the results is given as the ratio of the sample inhibition value/cutoff inhibition value (S/C), while the fluorescence, and the results is given as the ratio of the sample inhibition value to the cutoff inhibition value. The S/C ratio >1.0 is recorded as positive by the processor [14].

The standard curves of OTC and TC were each constructed to determine the detection limit of each. As shown in Fig. 1, the detection limits of OTC and TC were less than 0.1 µg/ml based on the S/C ratio of 1.0 in the assay system. The standard curves of the OTC and TC showed linear regression between 0.05 and 0.5 µg/ml (OTC, R²=0.989; TC, R²=0.965). Okerman et al. [14] determined different antibiotic residues in bovine and in porcine kidneys by solid-phase fluorescence immunoassay. The ranges of S/C ratios from spiked samples spiked with 300 µg/kg tetracycline antibiotics were 1.77–1.93 for TC and 1.55–2.05 for OTC. In our study, the range of ratios for TC and OTC were similar to that of study described above.

Recovery of 0.1 and 0.5 µg/ml of OTC and TC spiked into non-treated muscle is shown in Table 1. All recoveries were more than 89% of the spiked value.

**Table 1. Recoveries of tetracyclines in muscle of flounder with SFFIA**

<table>
<thead>
<tr>
<th>Spiked concentration (mg/kg)</th>
<th>OTC</th>
<th></th>
<th></th>
<th>TC</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S/C ratio²</td>
<td>Recovery³</td>
<td></td>
<td></td>
<td>S/C ratio²</td>
<td>Recovery³</td>
</tr>
<tr>
<td>0.1</td>
<td>0.973 ± 0.029</td>
<td>92.8</td>
<td></td>
<td></td>
<td>0.922 ± 0.023</td>
<td>89.5</td>
</tr>
<tr>
<td>0.5</td>
<td>1.730 ± 0.040</td>
<td>90.9</td>
<td></td>
<td></td>
<td>1.666 ± 0.049</td>
<td>89.1</td>
</tr>
</tbody>
</table>

a) S/C ratio is the ratio of the sample inhibition value to the cutoff inhibition value.

b) Recovery obtained from the formula, (S/C ratio of spiked muscle/S/C ratio of standard solution) × 100.

c) S/C ratio is the ratio of the sample inhibition value to the cutoff inhibition value.

The analytical results of OTC and TC in muscles are shown in Table 2. All of OTC samples showed positive results (S/C ratio ≥1.0) after the 1st, 2nd and 3rd day of withdrawal. In the analytical results of TC samples, 3 of 5 samples were positive after the 3rd day of withdrawal. After the 5th day of withdrawal, all of both OTC and TC samples showed negative reaction (S/C ratio ≤0.1), and were believed to decrease under 0.1 µg/ml.

Hah et al. [4] studied the development of simultaneous analytical method for sulfonamide and tetracycline residues in fishes, and investigated recovery rates after spiked at the concentration of 0.1, 0.3, 0.6 and 1.0 µg/ml of OTC and TC in muscles. The range of recovery rate for OTC and TC was 65.8–73.4% and 51.4–53.2%, respectively. Lee et al. [7] studied to analyze tetracycline antibiotics using High-Performance Liquid Chromatography (HPLC) for fishery products, and examined recovery rates of tetracyclines in flounder muscle. At the concentration of 0.1 µg/ml, recovery rates of OTC and TC were 100%, and at the concentration of 0.1 µg/ml, recovery rate of OTC and TC were 98 and 97%, respectively. In the research by Rupp et al. [21], salmon tissue was fortified with 0.10, 0.25, 0.50, 0.75, and 10 µg/ml OTC, and the recoveries were 84, 76, 70, 76, and 85%, respectively. Also Pena et al. [17] reported that the recovery rates of OTC and TC from salmon muscle tissue fortified at 9.05, 0.1 and 0.2 µg/ml levels, ranged from 83.9
to 93.4%. In our study, the recovery rates of OTC and TC were higher than results of the research carried out Hah et al. and lower than those of the study carried out Lee et al., and were similar to those in the research carried out Pena et al. It was assumed that the different results of recovery rates were depended on experimental conditions and methods of sample extraction.

Jung and Kim [5] carried out a comparative study of the detectable methods of residual OTC in flounder muscle with simplified screening test after oral administration to flounders at a dose rate of 100 mg/kg, and analyzed it in the muscle with modified EEC 4-plate method. The analytical results showed positive reaction until the 20th day after administration. Malvisi et al. [11] investigated tissue distribution and residue depletion of OTC in sea bream after oral administration at a concentration of 7.5 g/kg feed for 14 days. OTC concentrations in sea bream muscle were declined under 0.1 µg/ml 20 days after treatment. With the consideration of the dosage and the route administered, the flounder muscle residue concentrations of OTC and TC in our study were similar or little lower than those of studies described above. In the above results, the depletion of OTC and TC administered dipping is faster than oral or intramuscular medication.

According to our results, the applied methods can be adopted easily for use to screen OTC and TC residues in tissue of farmed fishes after minimal sample preparation. It is thinkable that the inspector may be able to apply this method to screen for tetracycline antibiotics in tissue of fishes on the place of shipment or on fish farm. Fishes that show positive results may be banned from shipping until retest results become negative before they are forwarded.

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