# Determination of Oxytetracycline in Raw and Cooked Channel Catfish by Capillary Electrophoresis†

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Oxytetracycline (OTC) residues exceeding 0.1 ppm tolerance level were detected by capillary electrophoresis (CE) in catfish fillets 18 h after oral feeding with 37.5, 75.0, and 150.0 mg OTC/kg in medicated feed for 10 days. The CE migration time of OTC was 10.9 min using a 24 cm × 25 μm capillary under 8 kV constant voltage. The mean OTC recovery rates in spiked catfish were 92.9% over the concentrations of 0.1−25 ppm. Cooking procedures (frying, baking, and smoking at 190 °C) could only reduce but not completely eliminate OTC residues in catfish fillets.

**Keywords:** *Oxytetracycline; capillary electrophoresis; catfish; cooking; frying; baking; smoking*

## INTRODUCTION

Oxytetracycline (OTC) is a broad-spectrum antibiotic approved by the Food and Drug Administration (FDA) for use in animal feeds to control bacterial infections. In catfish aquaculture, OTC is administered to catfish at 50 mg/kg fish/day for 10 consecutive days in medicated feed to control bacterial hemorrhagic septicemia caused by *Aeromonas liquifaciens* and *A. hydraphila* and disease by *Pseudomonas liquifaciens* (Ruth and Reed, 1991). Since OTC residue in catfish muscle may pose a potential health threat, such as allergic reactions in hypersensitive individuals, the FDA has established a 0.1 ppm (mg/kg) tolerance level in catfish tissue and a 21-day withdrawal period following OTC treatment (Ruth and Reed, 1991).

Capillary electrophoresis (CE), designed for separation of proteins, DNA, and small neutral or charged molecules, has been used to separate and characterize OTC, either pure or in milk (Chen and Gu, 1995; Croubels et al., 1994; Tavares and McGuffin, 1994; White et al., 1993; Zhang et al., 1992). High pressure for a few minutes has been used to purge the CE capillary, thereby shortening the run time for separation. Since less sample volume is used for CE analysis compared to high-performance liquid chromatography (HPLC), a greatly reduced amount of organic solvent is required, which improves the handling and disposal cost for solvent waste. High-voltage CE was also developed to accelerate separation of compounds from a mixture. Furthermore, CE cartridges of different diameters and lengths can easily be assembled in the laboratory to improve the efficiency of the instrument and separation.

Despite these many advantages, CE has not been widely applied to analyze drug residues in animal tissues. The objective of this study was to apply CE analysis for determining OTC residues in raw and cooked channel catfish dosed at 37.5, 75.0, and 150 mg OTC/kg for 10 days, and to determine if cooking would reduce OTC to near zero levels when detectable OTC was present in raw fillets.

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## MATERIALS AND METHODS

**Chemicals**. HPLC grade methanol was purchased from Baxter (McGaw Park, IL). Water used for CE analyses was distilled and further purified using a Photronix Water System (reagent grade; Photronix Corp., Medway, MA).

OTC stock solution was prepared by dissolving 27.62 mg OTC hydrochloride (containing 905 μg oxytetracycline/mg; Sigma Chemical Company, St. Louis, MO) in a 25-mL volumetric flask with methanol. A spiking solution of 100 μg/mL was prepared by diluting the stock solution with methanol. Standard OTC solutions (0.1, 0.25, 0.5, 1.0, 2.5, 5.0, 10.0, 20.0, and 25.0 μg/mL) for CE analysis were prepared by diluting the spiking solution with methanol and 1 M HCl (1:1, v/v).

**Fish Samples and Cooking Procedures.** Channel catfish fillets used as blanks and for spiking to determine recovery of OTC were purchased from a local seafood store. OTC-treated channel catfish at 37.5, 75.0, or 150.0 mg/kg of body weight daily for 10 days were provided by Dr. Wilmer A. Rogers of the Department of Fisheries and Allied Aquacultures, Auburn University (Fisheries Experiment Station, Auburn, AL). The treated catfish had an average body weight of 0.87 kg (SD = 0.05) and length of 40.6 cm (SD = 0.2). The cultivation of these catfish, preparation of the feed and medicated feed, and feeding conditions were all described previously (Du et al., 1997). Catfish were collected 18 h after the last medicated feeding in order to maintain OTC residues in fillets. One matched fillet from each fish was analyzed raw for OTC residue and the other was cooked and analyzed. Fillets from each fish were wrapped in aluminum foil, placed in zip-lock plastic bags, kept under ice, and transported to the University of Georgia, Athens, for cooking.

Four different cooking procedures previously described by Du et al. (1997) were applied to cook catfish fillets. These were frying in a deep-fat fryer (Colinco Fish Cooker CC-10, Damark International, Minneapolis, MN) of breaded (House Autry, House Autry Mills Inc., Newton Grove, NC) fillets in canola oil (Crisco, Proctor & Gamble, Cincinnati, OH) at 190 °C for about 7−10 min until golden brown; frying in canola oil at 190 °C for about 7−10 min of fillets injected with a 6% polyphosphate solution (Lem-O-fos, Rhone-Poulnec Basuc Chemical Co., Shelton, CT); baking of fillets at 190 °C in an oven (CPS 127, 68.6 cm Single Electric, Dacor, Pasadena, CA) for about 45 min; and hot smoking for 2 h (140 °C wet weight basis [wb], 160 °C dry weight basis [db] for 1 h and 180 °C wb, 200 °C db for 1 h) of fillets already soaked in a 25% NaCl solution (EM Science, Gibbstown, NJ) at 10 °C for about 1 h and air-dried. All cooked fillets were labeled, wrapped in aluminum foil, and placed in zip-lock plastic bags. After the samples were frozen at −23 °C, they were shipped along with the raw fillet samples on dry ice to the Food Science and Human Nutrition Department, University of Florida for OTC residue analysis.

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**Measurement of Moisture Contents.** The moisture contents of catfish fillets were determined by an AOAC (1990) oven method 950.46 using approximately 3-g ground samples in predried 55 mm aluminum moisture dishes for drying under vacuum for 18 h at 95−105 °C. The moisture dishes were transferred to a desiccator for cooling, then weighed. The samples were dried another 2 h, cooled, and weighed again. This process was repeated until sample weights became constant. The loss in weight in duplicate samples was used to calculate moisture content.

**Extraction of OTC from Fish Samples.** OTC-treated fillets (raw and cooked), spiked samples (at 0.05, 0.1, 0.2, 0.5, 1.0, 2.0, and 5.0 ppm) used to check daily recovery, and blanks were extracted using the previously described method of Du et al. (1995). Briefly, catfish fillets were chopped into a fine mince with a knife. For baked and breaded fried samples, bread was removed prior to analysis. Five-gram minced samples were manually mixed in 50-mL centrifuge tubes with 2 mL of 50% trichloroacetic acid (TCA), 30 mL of 1 M HCl, and 0.5 g of EDTA (disodium) for 3 min with a glass rod, and then vortex-mixed at high speed for 30 s. Samples were centrifuged at 920*g* for 10 min, and the supernatant filtered through a folded Whatman No. 1 filter paper. The pellet was then re-extracted with 1 mL of 50% TCA and 15 mL of 1 M HCl. All filtrates were pooled in 50-mL glass tubes, and passed through Sep-Pak $C_{18}$ cartridges (Waters Associates, Milford, MA) attached to a PrepTorr vacuum box (Fisher Scientific, Atlanta, GA) for OTC adsorption. The cartridge was washed with 10 mL methanol and then 10 mL water. The retained OTC was eluted from the cartridge with 10 mL methanol. After 1 mL 0.1% aqueous dithiothreitol was added to the eluant, the sample was concentrated to about 0.5 mL under a stream of nitrogen in a 40 °C water bath. The volume was readjusted to 1 mL with 1 M HCl and vortex-mixed for 1 min. The suspension was filtered through a 0.45 $\mu$m filter and the filtrate was used for CE analysis.

**OTC Determination by CE.** CE was performed on a BioFocus 2000 Capillary Electrophoresis System (Bio-Rad, Hercules, CA) using a coated capillary cartridge of 24 cm × 25 $\mu$m i.d. The cartridge temperature was maintained at 25 °C. Before injection, the capillary cartridge was rinsed with 0.1 N NaOH for 2 min, then water for 3 min, and finally the running phosphate buffer (0.2 M, pH 2) for 3 min. An 8.4 nL aliquot of sample was injected into the capillary by low-pressure injection. A constant voltage at 8 kV was applied for separation of OTC. Detection was performed by monitoring the absorbance at 265 nm. Data were stored in a personal computer and processed using a BioFocus integrator (Bio-Rad) program.

During determination with CE, OTC standards were analyzed together with test catfish samples (blank, spiked, and treated raw and cooked). During analysis, a standard solution was also injected intermittently along with test samples to check consistency. Each sample was injected twice. Peak areas of OTC standard solutions were used to prepare a standard curve. From these standard curves, OTC concentrations in test samples were calculated. At the end of each experiment, the CE was run with a shut-down cycle.

**Statistical Analysis.** Data for OTC residues in raw and cooked catfish treated with different OTC levels were analyzed using analysis of variance (SAS Institute, Inc., 1989). Duncan's multiple range test was used to obtain pairwise comparisons among sample means. Significance was based on a $P = 0.05$ level.

## RESULTS AND DISCUSSION

**Moisture Content of Fish Fillets.** Previously reported data (Du et al., 1997) showed that cooking caused a significant ($P < 0.05$) reduction of mean moisture content of catfish fillets from 75.5 (SD = 3.1) to 68.6% (SD = 4.6). After they were cooked, the smoked fillets had the lowest moisture content (63.8 ± 4.0%), while the fried fillets of phosphate-injected samples had the highest (72.6 ± 2.1%). The loss of moisture in cooked fillets was related to the final time and temperature used for cooking.

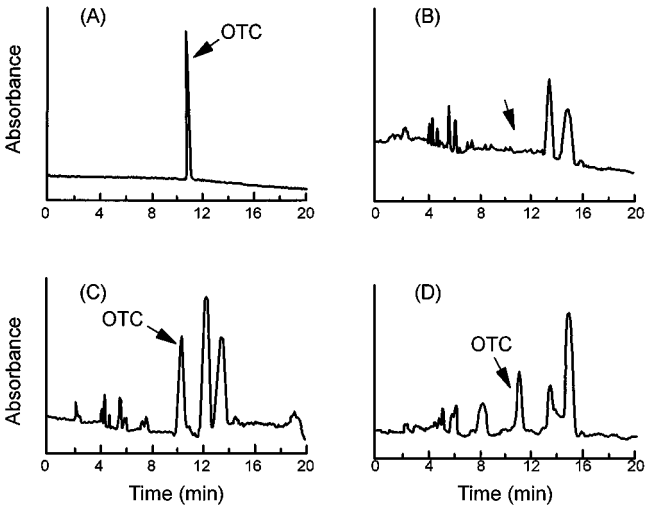


**Figure 1.** Typical electrophoretograms of (A) 1.0 ppm OTC standard solution, and muscle extracts of (B) blank catfish, (C) untreated catfish spiked with 1 $\mu$g OTC/g, and (D) OTC-treated raw catfish.

**Table 1. Recovery of Oxytetracycline from Spiked Catfish Muscle Using CE**

| fortification (ppm) | concn found (ppm) | av recovery (% mean ± SD, $n = 3$) | CV (%) |
|---|---|---|---|
| 0.1 | 0.094 | 94.5 ± 3.3 | 3.5 |
| 0.5 | 0.468 | 93.5 ± 3.5 | 3.7 |
| 1 | 0.939 | 93.9 ± 3.6 | 3.8 |
| 5 | 4.48 | 89.6 ± 2.3 | 2.6 |
| | mean | 92.9 ± 3.2 | 3.4 |

**CE Determination of OTC Residues in Raw and Cooked Catfish.** The CE electrophoretograms of OTC standard and muscle extracts of blank, OTC-spiked, and treated raw catfish (Figure 1) showed no interference with OTC peak in treated catfish muscle. The migration time of OTC was 10.9 ± 0.28 min ($n = 125$) under a constant voltage of 8 kV using a 24 cm × 25 $\mu$m capillary cartridge. Very high linear relationships ($R^2 > 0.999$, $n = 10$) occurred with peak areas and concentrations over 0.1−25 ppm OTC standard solutions. OTC detection limits were 0.42 ng for a standard solution (0.05 $\mu$g/mL, 8.4 nL injection volume) and 0.05 ppm for catfish extract. The reproducibility of the CE method was verified by determining the intra- and inter-assay variations of OTC in fortified fillets. The average coefficient of variation (CV) for the intra-assay was 3.2%, and 5.0% for inter-assay. A 92.9% recovery rate was achieved from OTC-spiked catfish muscle over the concentration range of 0.1−5 ppm (Table 1).

OTC residues exceeding the 0.1 ppm tolerance level were detected in raw catfish 18 h after oral administration of 37.5, 75.0, or 150.0 mg OTC/kg of fish for 10 days (Table 2). No corrections for recovery loss were made with these data. The differences in feed uptake and metabolism of OTC among catfish might contribute to inter-fish variation in OTC residues in each treatment group. Inter-fish variability in muscle content of OTC (Aoyama et al., 1991), penicillin (Kitts et al., 1994), and sulfadimethoxine (Pleasance et al., 1991; Kitts et al., 1995; Walisser et al., 1990) also occurred with salmon. This wide range of differences in individual OTC residues makes interpretation of the significance of results more difficult, although catfish receiving 150 mg OTC/kg had a higher ($P < 0.05$) residue level (1.81 ±

**Table 2. Corrected OTC Residues (ppm) on Dry Weight Basis of Raw and Cooked Catfish Fillets**

| | OTC residue (mean[a] ± SD; $n = 6$) | | |
|---|---|---|---|
| | 37.5 mg/kg | 75.0 mg/kg | 150.0 mg/kg |
| prefried, raw | $0.36 \pm 0.34$ | $0.48 \pm 0.37$ | $1.67 \pm 0.75$ |
| fried, cooked | $0.16 \pm 0.19^{AB}$ | $0.32 \pm 0.23^{AB}$ | $0.46 \pm 0.36^{A,a}$ |
| prebaked, raw | $0.74 \pm 0.75$ | $0.39 \pm 0.19$ | $2.38 \pm 2.70$ |
| baked, cooked | $0.05 \pm 0.09^{B}$ | $0.17 \pm 0.17^{AB}$ | $1.12 \pm 1.81^{A}$ |
| pre$PO_4$-injected-fried, raw | $0.36 \pm 0.58$ | $0.74 \pm 0.40$ | $1.50 \pm 0.46$ |
| $PO_4$-injected-fried, cooked | $0.25 \pm 0.18^{A}$ | $0.39 \pm 0.33^{A}$ | $0.68 \pm 0.36^{A,a}$ |
| presmoked, raw | $0.04 \pm 0.05$ | $0.33 \pm 0.19$ | $1.69 \pm 2.71$ |
| smoked, cooked | $0.03 \pm 0.04^{B}$ | $0.08 \pm 0.07^{B,a}$ | $0.34 \pm 0.28^{A}$ |

[a] Cooked means in the same dose level bearing a superscript a when compared to raw fish counterpart were significantly different ($P = 0.05$). Means within the same dose level bearing the same superscript capital letter are not significantly different ($P = 0.05$) following different cooking method.

1.82 ppm, Table 2, precooked samples) than those receiving 75.0 ($0.48 \pm 0.32$ ppm) or 37.5 mg/kg ($0.38 \pm 0.53$ ppm). Since one of our objectives was to investigate effects of cooking on OTC residues, the OTC-treated catfish were purposely harvested 18 h post oral feeding in order to maintain OTC residues in raw fillets. No OTC residue was detected in catfish muscle 48 h after oral administration of OTC even at 200 mg/kg for 10 days (Fribourgh et al., 1969). Apparently, the short withdrawal time between the last feeding of OTC-medicated feed and harvesting affected OTC residues in catfish muscle.

Comparisons between raw and cooked fillets for each cooking method at each feeding level showed that cooking of catfish caused reduction in OTC residues (Table 2). After fillets were cooked, the 150 mg/kg group, in general, had higher residue levels than the 37.5 mg/kg group. Except for the 150 mg/kg group, baking and smoking were generally more effective than frying in reducing OTC residues in fillets. The longer cooking time used for baking and smoking apparently contributed to greater losses of OTC in cooked fillets. Catfish fillets fried for 7−10 min had an internal temperature of 71 °C (Du et al., 1997). OTC residues were therefore less effectively reduced by frying than by baking or smoking. Kitts et al. (1992) also reported that OTC was relatively heat stable in salmon tissue under ordinary frying procedures. However, the individual differences in feed uptake and metabolism of OTC also made it very difficult to clearly conclude that fillets cooked by a specific method had a lower level of OTC residues than those cooked by other procedures.

In addition to differences in OTC pharmacodynamics in catfish muscle and the cooking temperatures, the shape and thickness of each fillet also affected heat penetration and distribution and hence degradation of OTC residues. Yonova (1971) showed that antibiotics in animal tissues could only be degraded by high temperatures maintained for considerable periods. Since such high temperatures were not attained and maintained in this cooking study, OTC residues remained in cooked fillets.

**Conclusion.** Similar to HPLC, CE allowed for detection of OTC residues exceeding the 0.1 ppm tolerance level in most of the raw catfish 18 h after feeding of 37.5, 75.0, or 150.0 mg OTC/kg in medicated feed for 10 days. Since less sample extract (in nanoliters) is used in CE analysis than in HPLC (in microliters), less solvent was used and less solvent waste produced using CE compared to HPLC. Thus, the CE method has great potential to replace HPLC for analysis of OTC residues in catfish and possibly other food items. Although the CE method was not applied in this study to detect degradation products of OTC following cooking, the results showed that ordinary cooking by frying, baking, and smoking could not completely eliminate high levels of OTC residues in catfish fillets.

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