Pharmacokinetics, tissue distribution, and metabolism of flumequine in channel catfish (Ictalurus punctatus)

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Abstract

The pharmacokinetics and metabolism of the fluoroquinolone drug flumequine (FLU) were examined after intravascular (1 mg/kg) or oral (5 mg/kg) dosing in channel catfish. Parent FLU concentrations in plasma declined slowly after intravascular dosing, with a half-life of 25 h. After oral dosing, FLU concentrations in plasma were highest (3.1 \textmu g/ml) at 14 h after dosing; absorption and elimination half-lives were 4.9 and 22 h, respectively. The oral bioavailability of FLU was 44%, based on normalized plasma data. Plasma protein binding of parent FLU was extensive, but saturable (88\%–55\% bound at 0.125–8.0 \textmu g/ml). After oral dosing with \textsuperscript{14}C-labeled FLU, radioactive residues were evenly distributed among the major tissues analyzed, with peak concentrations occurring at 12–24 h. Residue concentrations were highest in liver (6.2 \textmu g/g) and lowest in muscle (1.8 \textmu g/g) at 24 h. Only parent FLU was found in muscle and was eliminated with a half-life of 26 h. FLU and its metabolites were recovered in urine and bile. Residues in bile consisted almost entirely of a taurine conjugate of FLU. In urine, the taurine conjugate and hydroxy-FLU metabolites were found, in addition to the parent compound. Published by Elsevier Science B.V.

Keywords: Flumequine; Pharmacokinetics; Metabolism; Channel catfish

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1. Introduction

Flumequine (FLU) is a fluoroquinolone drug used in aquaculture primarily for treatment of Gram-negative bacterial infections in fish. Its use in European aquaculture is well documented, particularly in the Atlantic salmon industry (Grave et al., 1996). Widespread application of FLU in the past has raised environmental and food safety concerns, with increased scrutiny and modifications in its aquatic applications. FLU is not approved for food fish use in the United States.

Pharmacokinetic and metabolism data are valuable in establishing drug dosage and withdrawal periods in food-producing animals and in developing suitable methods for monitoring residues in animal products. In fish, these data exist for FLU in several freshwater and marine species (Boon et al., 1991; Rogstad et al., 1993; Elema et al., 1994,1995; Sohlberg et al., 1994,1996; Van der Heijden et al., 1994; Hiney et al., 1995; Martinsen and Horsberg, 1995; Samuelsen and Ervik, 1997). In general, FLU is moderately absorbed when administered orally in fish and residues are well distributed in the tissues. FLU is also absorbed during bath treatment (O’Grady et al., 1988; Samuelsen and Lunestad, 1996). Elimination of FLU in fish is generally slow, but significant species differences exist (Van der Heijden et al., 1994). Metabolism of FLU in fish also appears slow; metabolite levels in tissues are typically low or undetectable (Haagsma et al., 1993; Van der Heijden et al., 1993,1994; Samuelsen and Ervik, 1997).

We describe the pharmacokinetics and metabolism of FLU in farm-raised channel catfish (Ictalurus punctatus). Distribution and elimination of residues in the tissues are characterized, with emphasis on the edible flesh. Metabolites of FLU in selected tissues and fluids are identified.

2. Materials and methods

2.1. Chemicals

[2,14C]-Flumequine (specific activity, 30.4 mCi/mmol) was custom synthesized by NEN Research Products (Boston, MA). Radiochemical and chemical purity were > 99%, as determined by liquid chromatography (HPLC) and thin-layer chromatography. Unlabeled FLU (> 99% purity by HPLC) was purchased from Sigma Chemical Co. (St. Louis, MO).

2.2. Animals

Channel catfish (Ictalurus punctatus) were obtained from a local farm, with no history of FLU treatment; mean body weight (±SD) of fish used in the study was 0.66 ± 0.14 kg. Animals were allowed to adapt to study conditions for at least 2 weeks while being fed a commercial catfish feed (Purina Mills, St. Louis, MO). At 1–2 days prior to surgery or dosing, animals were placed in stainless steel cages (Plakas et al., 1994) and held in 285-Liter Living Stream Units (Frigid Units, Toledo, OH) with activated carbon filtration. Mean water temperature during the study was 24°C and pH was 7.6.
2.3. Surgical procedures

For pharmacokinetic evaluations, animals were catheterized for serial sampling of blood, as described by Stehly and Plakas (1993). Animals were allowed to recover for 1–2 days before dosing. To examine renal excretion, the urinary bladders of fish were cannulated (Plakas et al., 1994). Urine flow was confirmed before dosing.

2.4. Dosing solutions

Intravascular dosing solution was prepared at a concentration of 2 mg/ml by using unlabeled FLU. To prepare 5 ml, 10 mg FLU was dissolved in 3.5 ml of 0.1 M Na₂CO₃ with sonication. The solution was adjusted to pH 9 with 1 N HCl and brought to volume with water. Oral dosing solutions (with or without radiolabel) were similarly prepared, but at a FLU concentration of 10 mg/ml. For radioactive solutions, portions of the unlabeled FLU were substituted with ¹⁴C-FLU to yield an activity level of 100 µCi/ml (10 µCi/mg). The pH of dosing solutions was re-adjusted before use to ensure complete solubilization of the drug. Dosing solutions were administered at a rate of 0.5 ml/kg body weight. FLU did not precipitate from solution when mixed with plasma during in vitro simulation of intravascular dosing.

2.5. Dosing and sampling

2.5.1. Pharmacokinetics

Intravascular doses of 1 mg/kg were administered through the catheters of five fish by using disposable syringes with blunted needles. Doses were followed with ~1 ml of heparinized saline. Clean syringes and needles with three-way stopcocks were attached. Blood specimens (0.25–0.3 ml each) were taken as previously described (Stehly and Plakas, 1993) at various intervals after dosing and were immediately packed in ice. Plasma was separated by centrifugation and stored at −80°C until analysis by HPLC.

Oral doses of 5 mg/kg were administered in gelatin capsules (size 00, Torpac, East Hanover, NJ) by gavage to anesthetized animals (n = 5). Each capsule contained 0.25 g of ground catfish feed onto which the dosing solution was dispersed. After dosing, fish were returned to their tanks and plasma specimens were collected as described above.

2.5.2. Tissue distribution and elimination

The tissue distribution and elimination of total drug residues were determined after oral dosing with ¹⁴C-FLU at 5 mg/kg. At each sampling time, five animals were stunned by cranial concussion and blood specimens were taken by syringe from the caudal vein. Fish were then euthanized by cervical dislocation and selected organs (liver, head kidney, trunk kidney, and spleen) were dissected, briefly rinsed with water, blotted, and weighed. Bile was taken from the gall bladder by syringe. Muscle fillets (skin removed) were taken from both sides of the fish and homogenized in a Waring blender. All tissues and fluids were stored at −80°C until analysis.
2.5.3. Renal excretion
  Cannulated animals were orally dosed with $^{14}$C-FLU at 5 mg/kg. Urine was continuously collected in polypropylene containers packed in ice. At various intervals after dosing, urine volumes were recorded and specimens were stored at $-80^\circ$C until analysis. Excretion data were compiled for three animals in which cannulae remained patent for 2 weeks after dosing.

2.5.4. Metabolism
  Selected tissues and fluids containing radioactive residues (as collected above) were examined for FLU metabolic profile. Plasma, muscle, liver, and kidney specimens collected at the 24-h sampling time provided sufficient levels of radioactivity for metabolite analysis. Equal volumes of bile or urine specimens were pooled and analyzed for each sampling time. Additional fish ($n = 3$) were orally dosed with unlabeled FLU (5 mg/kg) to provide non-radioactive residues for mass spectrometric characterization of metabolites.

2.6. Analytical methods

2.6.1. HPLC
  Procedures for extraction and HPLC analysis of parent FLU in muscle and plasma were described previously (Plakas et al., 1999). Briefly, FLU was extracted with acidified methanol (glacial acetic acid-methanol, 2 + 98), and extracts were cleaned-up on C$_{18}$ solid-phase extraction (SPE) columns. FLU concentrations were determined by using a C$_{18}$ HPLC column, an isocratic mobile phase (glacial acetic acid–water–acetonitrile, 2 + 48 + 50), and fluorescence detection (excitation, 325 nm; emission, 360 nm). Standard curves were prepared by fortification of control tissues with FLU.

  Metabolic profile of $^{14}$C-FLU in selected tissues and fluids was determined by HPLC operated under gradient mobile phase conditions. Extraction and clean-up procedures for total residues in tissues were the same as described above for parent FLU. Recoveries of total radioactivity in these tissues were $\geq 90\%$. Residues in urine were concentrated and cleaned-up by SPE. Five-milliliter aliquots of pooled urine were applied to 6-ml C$_{18}$ SPE columns and, after elution and evaporation (as per tissue procedure), residues were re-solubilized in 0.5 ml of initial mobile phase for analysis. Bile was diluted (1:10) with initial mobile phase, filtered, and injected directly onto the HPLC column. Mobile phase reservoirs were: (A) glacial acetic acid:water (2 + 98); (B) glacial acetic acid:acetonitrile (2 + 98). Gradient conditions were: 70% A for 2.5 min, ramp to 50% A at 15 min, hold at 50% A for 10 min, return to initial conditions (70% A), and re-equilibrate for $\geq 15$ min before the next injection. A fraction collector was used to monitor radioactivity in HPLC column effluents (0.2-min fractions).

2.6.2. Liquid chromatography / mass spectrometry (LC / MS)
  HPLC effluent fractions from non-radioactive tissue or fluid specimens were collected based on metabolite peak retention times as identified in radioactive samples. Peak fractions from several HPLC injections were pooled and evaporated by using a vacuum centrifuge. Dried residues were re-dissolved in 0.5 ml of methanol for MS analysis.
The LC/MS system consisted of a Hewlett-Packard (Palo Alto, CA) Model 1050 LC pump and a Finnigan (San Jose, CA) Model TSQ-7000 triple-quadrupole mass spectrometer with a standard Finnigan electrospray (ESI) ion source. All chromatography was performed on a YMC (Wilmington, NC) J-sphere ODS-M80 LC column (2 × 250 mm). The mobile phase flow rate was 0.2 ml/min and all other buffers and gradients were the same as those used to prepare the metabolite fractions. Nitrogen was used as a nebulizing gas and the ion source capillary temperature was 230°C. For full scan MS experiments, the instrument was scanned over the range of 150–700 amu at 1 s/scan. For MS/MS experiments, the instrument was scanned from 15–380 amu at 1 s/scan. Argon was used as the collision gas and collision energy was 40 V.

2.6.3. Liquid scintillation counting (LSC)

Total 14C contents in tissues, fluids, and HPLC effluent fractions were determined by LSC, as previously described (Plakas et al., 1998). Radioactivity in tissues or fluids was expressed in units of concentration (μg equiv. of FLU/g or ml) or as a percentage of the administered dose. With no practical means of solubilizing bone for LSC, caudal vertebrae specimens (~0.5 g) from each fish were extracted twice with 4 ml acidified methanol by shaking overnight at room temperature in a mechanical shaker. Methanolic extracts were combined and aliquots were analyzed by LSC.

2.6.4. Plasma protein binding

Plasma protein binding of parent FLU was determined by ultrafiltration (Centrifree Micropartition System, Amicon, Beverly, MA). 14C-FLU was added to control plasma at concentrations ranging from 0.125 to 8 μg/ml. Duplicate aliquots (0.4 ml) of spiked plasma were added to the ultrafilters and centrifuged at 1000 × g for 1 h (25°C). Entire ultrafiltrates were analyzed for 14C-FLU contents by LSC, representing unbound drug. Values were corrected for non-specific absorption of unbound drug to the ultrafiltration apparatus (~6%). Non-specific absorption was determined by the corresponding analysis of control plasma ultrafiltrates spiked with 14C-FLU. Additionally, plasma specimens collected from fish dosed orally with 14C-FLU (as described above) were analyzed for protein binding of total radioactive residues.

2.6.5. Pharmacokinetics

The computer program WinNonlin (Scientific Consulting, Cary, NC) was used to derive pharmacokinetic values for parent FLU concentrations in plasma. Oral bioavailability was calculated from the areas under the plasma concentration-time curves, normalized for dose.

3. Results

3.1. Pharmacokinetics

Parent FLU levels in plasma declined slowly after intravascular dosing and were measurable (~0.01 μg/ml) for up to 168 h (Fig. 1). Elimination half-life was 25 h.
Fig. 1. Mean parent FLU concentrations in plasma after intravascular dosing at 1 mg/kg (■) or oral dosing at 5 mg/kg (○) in channel catfish (n = 5).

(Table 1). Other pharmacokinetic parameters (Table 1) indicate moderate distribution of FLU outside of the vascular system ($V_{ss}$, 527 ml/kg), and slow clearance ($Cl_h$, 14.9 ml/h/kg).

After oral dosing, parent FLU was measurable in plasma within 15 min (Fig. 1). Peak plasma concentration was 3.1 μg/ml at 14 h. Absorption and elimination half-lives were 4.9 and 22 h, respectively (Table 1). The bioavailability of FLU was 44%. FLU levels were not quantifiable beyond 168 h.

3.2. Plasma protein binding

Plasma protein binding of 14C-FLU in spiked plasma was extensive, but saturable, declining from 88% at 0.125 μg/ml to 55% at 8 μg/ml (Fig. 2). Similar values were

<table>
<thead>
<tr>
<th>Intravascular (1 mg/kg)</th>
<th>Oral (5 mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parameter</td>
<td>Unit</td>
</tr>
<tr>
<td>$t_{1/2}el$</td>
<td>h</td>
</tr>
<tr>
<td>AUC</td>
<td>μg·h/ml</td>
</tr>
<tr>
<td>$Cl_h$</td>
<td>ml/h/kg</td>
</tr>
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<td>$V_{ss}$</td>
<td>ml/kg</td>
</tr>
<tr>
<td>MRT</td>
<td>h</td>
</tr>
<tr>
<td>$F$</td>
<td>%</td>
</tr>
</tbody>
</table>

Pharmacokinetic parameter abbreviations: $t_{1/2}el$, elimination half-life; AUC, area under the plasma concentration-time curve; $Cl_h$, total body clearance; $V_{ss}$, apparent volume of distribution at steady-state; MRT, mean residence time; $t_{1/2}abs$, absorption half-life; $C_{max}$, maximum plasma concentration; $t_{max}$, time to $C_{max}$; $F$, fraction of the oral dose available systemically.
found for residual $^{14}$C in plasma of orally dosed animals, depending on concentration or time after dosing (data not shown). For example, mean binding values were 67% at 24 h (peak $^{14}$C levels) and 89% at 168 h.

### 3.3. Tissue distribution and elimination

After oral dosing with $^{14}$C-FLU, total residue concentrations in the tissues were highest at 12–24 h (Table 2). At 24 h, liver had the highest concentrations (6.2 µg/g) and muscle had the lowest (1.8 µg/g) of any tissues analyzed. Concentrations in plasma, trunk kidney, head kidney, and spleen were similar, ranging from 3.3 to 4.4 µg/g at 24 h. Rates of elimination of radioactive residues were also similar between individual tissues. In muscle, $^{14}$C concentrations declined below the limit of determination.

![Fig. 2. Plasma protein binding of FLU as determined in vitro.](image)

Table 2

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Time after dosing (h)</th>
<th>3</th>
<th>6</th>
<th>12</th>
<th>24</th>
<th>72</th>
<th>168</th>
<th>336</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bile</td>
<td></td>
<td>0.48±0.24</td>
<td>6.30±7.52</td>
<td>12.2±6.18</td>
<td>33.9±15.3</td>
<td>119±71.1</td>
<td>91.0±34.9</td>
<td>82.4±27.7</td>
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<tr>
<td>Liver</td>
<td></td>
<td>2.15±2.12</td>
<td>4.62±2.91</td>
<td>5.46±2.01</td>
<td>6.15±1.24</td>
<td>1.77±0.74</td>
<td>0.34±0.20</td>
<td>0.08±0.04</td>
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<tr>
<td>Plasma</td>
<td></td>
<td>1.71±0.46</td>
<td>3.16±0.96</td>
<td>4.00±1.97</td>
<td>4.10±0.89</td>
<td>3.00±1.20</td>
<td>0.49±0.44</td>
<td>0.02±0.02</td>
</tr>
<tr>
<td>Trunk kidney</td>
<td></td>
<td>0.95±0.24</td>
<td>2.92±1.35</td>
<td>3.78±1.54</td>
<td>4.41±1.16</td>
<td>1.54±0.52</td>
<td>0.25±0.17</td>
<td>0.05±0.02</td>
</tr>
<tr>
<td>Head kidney</td>
<td></td>
<td>0.75±0.27</td>
<td>2.42±1.12</td>
<td>3.80±1.75</td>
<td>3.37±0.82</td>
<td>1.07±0.38</td>
<td>0.22±0.16</td>
<td>0.07±0.03</td>
</tr>
<tr>
<td>Spleen</td>
<td></td>
<td>0.70±0.27</td>
<td>2.42±1.16</td>
<td>3.35±1.26</td>
<td>3.34±0.78</td>
<td>1.07±0.46</td>
<td>0.24±0.18</td>
<td>0.11±0.06</td>
</tr>
<tr>
<td>Skin</td>
<td></td>
<td>0.42±0.12</td>
<td>1.32±0.74</td>
<td>1.84±0.73</td>
<td>2.18±0.72</td>
<td>0.84±0.20</td>
<td>0.26±0.16</td>
<td>0.09±0.05</td>
</tr>
<tr>
<td>Muscle</td>
<td></td>
<td>0.28±0.12</td>
<td>1.03±0.68</td>
<td>1.80±0.91</td>
<td>1.84±0.47</td>
<td>0.42±0.19</td>
<td>0.07±0.06</td>
<td>-b</td>
</tr>
</tbody>
</table>

*Values are the mean ± s.d. of five animals.

bBelow the limit of determination (< 0.005 µg equiv./g).
tion by LSC ($< 0.005 \mu g/g$) at 336 h. In skin, concentrations were initially similar to those in muscle, but were eliminated more slowly. Residue levels in bone, as estimated by solvent extraction, were 0.11 $\mu g/g$ at 3 h, 0.66 $\mu g/g$ at 24 h, and 0.03 $\mu g/g$ at 336 h (data not shown).

Parent FLU concentrations in muscle, as determined by HPLC (Fig. 3), were nearly identical to total residue concentrations, by LSC (Table 2). Parent FLU levels were 1.8 $\mu g/g$ at 24 h and 0.07 at 168 h (Fig. 3). Parent FLU was not quantifiable ($< 0.01 \mu g/g$) beyond 168 h. Estimated half-life for elimination of FLU in muscle was 26 h.

Fig. 3. Parent FLU concentrations in muscle after oral dosing at 5 mg/kg in channel catfish. Values are the mean ± s.d. of five animals.

Fig. 4. Renal excretion of FLU and its metabolites (total residues) after oral dosing with $^{14}$C-FLU at 5 mg/kg in channel catfish: ■, concentration ($\mu g$ equiv./ml); •, cumulative % dose.
3.4. Excretion

Residues were highly concentrated in bile, relative to the tissues. Total residue concentrations increased rapidly from 0.48 μg/ml at 3 h to 119 μg/ml at 72 h, and then declined slowly to 82 μg/ml at 336 h (Table 2). At peak levels (72 h), the amounts of 14C contained in the gall bladder represented 3–4% of the administered oral dose.

In urine, total residue concentrations were highest (0.88 μg/ml) at the 12–24 h sampling interval (Fig. 4). By 168 h, 14C levels in urine were 0.04 μg/ml and at 336 h, <0.01 μg/ml. Cumulatively, 4.9% of the oral dose was excreted in urine over the 2-week sampling period.

3.5. Metabolism

Only parent FLU was found in muscle and plasma after oral dosing with 14C-FLU, under our conditions of analysis. In bile, parent FLU was a minor component at the

![Radiochromatograms of bile and urine collected from channel catfish after oral dosing with 14C-FLU at 5 mg/kg.](image)

Fig. 5. Radiochromatograms of bile and urine collected from channel catfish after oral dosing with 14C-FLU at 5 mg/kg.
earlier sampling times (e.g., 12% of total residues at 24 h), but was not detectable beyond 72 h. Residues in bile were mostly composed of a single, polar metabolite with a retention time of ~13 min (Fig. 5). The molecular weight of this metabolite was 368, by LC/MS. This finding, along with a significant fragment ion at m/z 80 (80% relative abundance) in the negative ion MS/MS spectrum, is consistent with the metabolite being a taurine conjugate of FLU.

In urine, four radioactive peaks were found (Fig. 5). Parent FLU comprised 72% of total radioactive residues at 3 h, 55% at 24 h, and 38% at 168 h. The taurine conjugate of FLU also was identified, comprising 11% of total residues at 3 h, 32% at 24 h, and 59% at 168 h. Two minor metabolites, which eluted as a doublet peak (retention time, 9.5–10 min) by HPLC, comprised <10% of total radioactivity. These minor metabolites were both identified as hydroxyl-FLU, and were probably structural isomers. Liver and kidney contained mostly (>80%) parent FLU at 24 h.

4. Discussion

Parent FLU levels declined slowly after intravascular and oral dosing, with elimination half-lives of 25 and 22 h, respectively, at 24°C. Much longer half-lives were reported in other freshwater species. Elimination half-lives in European eel, common carp, and African catfish, were 451, 104, and 59.5 h, respectively, at 24°C (Van der Heijden et al., 1994). Boon et al. (1991) reported a terminal elimination half-life of 256 h in European eel at 23°C. In freshwater rainbow trout, FLU half-life in plasma was 569 h at 3°C and 137 h at 13°C (Sohlberg et al., 1994). In seawater, elimination of FLU in fish is generally more rapid than in freshwater, even at lower temperatures. In Atlantic salmon held in saltwater, FLU half-lives were 21–23 h in studies conducted at 5–10°C (Rogstad et al., 1993; Elema et al., 1994; Martinsen and Horsberg, 1995). Elema et al. (1995) found half-lives of 30–40 h in Atlantic salmon at 6–8°C. In Atlantic salmon smolts, FLU half-life was 170 h in freshwater compared with 140 h in seawater, at 11°C (Hiney et al., 1995). Sohlberg et al. (1996) reported elimination half-lives of 67 and 38 h in cannulated and non-cannulated Atlantic salmon, respectively, after intravascular dosing at 11°C. In Atlantic halibut, terminal half-life of FLU was 43 h, at 9°C (Samuelsen and Ervik, 1997).

A bi-exponential equation provided the best fit of experimental data after intravascular dosing, but with low confidence in the initial (distribution) phase. A mono-exponential equation could also be applied with virtually no change in those pharmacokinetic values reported in Table 1. Plasma protein binding of FLU was extensive and saturable in the range of concentrations observed in vivo, which possibly influenced FLU distribution kinetics under our dosing conditions. Boon et al. (1991) found concentration-dependent changes in plasma protein binding of FLU in eel, although binding values (30–40%) were much lower than in catfish. Other processes (e.g., metabolism, excretion) may also be non-linear with respect to dose (or concentration) of FLU.

Pharmacokinetic values for FLU in channel catfish indicate moderate distribution outside of the vascular system (Vss, 0.527 l/kg) and slow clearance (Clss, 0.015 l/h/kg). In studies with Atlantic salmon, Vss values ranged from 1 to 4 l/kg and Clss
from 0.005 to 0.2 l/h/kg (Rogstad et al., 1993; Sohlberg et al., 1994, 1996; Elema et al., 1995; Martinsen and Horsberg, 1995). In Atlantic halibut, $V_{ss}$ and $Cl_{h}$ values were 2.3 l/kg and 0.052 l/h/kg, respectively (Samuelsen and Ervik, 1997). Although not reported in the latter studies, plasma protein binding may be an important variable influencing distribution and clearance of FLU.

After oral dosing in channel catfish, peak levels ($C_{max}$, 3.1 µg/ml) of parent FLU in plasma occurred at 14 h ($t_{max}$), with an absorption half-life of 4.9 h. Bioavailability of FLU was 44%. Oral absorption is also fairly rapid, but incomplete, in other fish species. However, species differences occur even under identical dosing conditions (Van der Heijden et al., 1994). In some freshwater species, peak concentrations were found within 2 h after dosing (Van der Heijden et al., 1994). In Atlantic salmon, plasma FLU levels typically peak at 12–24 h after oral dosing, with a bioavailability of 35–55% (Rogstad et al., 1993; Elema et al., 1995; Martinsen and Horsberg, 1995; Sohlberg et al., 1996). In Atlantic halibut, $t_{max}$ was 20 h and bioavailability was 31% (Samuelsen and Ervik, 1997). Absorption of FLU may be lower in seawater by its complex with divalent cations, as found with the fluoroquinolone drug difloxacin (Elston et al., 1994). FLU is very stable in marine sediments, and its application and incomplete absorption in medicated feeds may have environmental consequences (Erikv et al., 1994; Samuelsen et al., 1994).

Total residue concentrations in the tissues of channel catfish were highest at 12–24 h after dosing and rates of elimination were generally similar. At 24 h, tissue:plasma concentration ratios ranged from 0.45:1 in muscle to 1.5:1 in liver, which were congruent with pharmacokinetic analyses suggesting moderate extravascular distribution of FLU. In Atlantic salmon, tissue penetration of FLU appears more extensive than that in catfish, with higher tissue:plasma concentration ratios (Rogstad et al., 1993; Elema et al., 1994) and larger volumes of distribution. In some fishes (e.g., Atlantic salmon, sea bream), FLU is highly concentrated in skin and especially bone, relative to muscle (Steffenak et al., 1991, 1994; Malvisi et al., 1997). These tissues represent a deep storage compartment prolonging overall elimination of FLU. From the food safety standpoint, FLU may be released from these sites during normal processing and cooking of the marketed product (Steffenak et al., 1994). In channel catfish, total residue concentrations in skin were initially comparable to those in muscle, but were eliminated more slowly. Residue levels in bone, as estimated by solvent extraction, were mostly lower than in muscle. Possibly, solvent extraction of bone underestimates total residue content (i.e., bound drug).

Parent FLU was the only residue detected in muscle of catfish and was eliminated with a half-life of 26 h at 22°C. By comparison, half-life of FLU in muscle of rainbow trout was 11 h at 14°C (Malvisi et al., 1994). In Atlantic salmon, half-life was 18 and 21 h after 6- and 8-day medication periods, respectively, at 8°C (Elema et al., 1994). In Atlantic salmon smolts, half-life in muscle was 81 and 41 h for fish held in freshwater and seawater, respectively (Hiney et al., 1995). In juvenile Atlantic halibut, FLU half-life in muscle was 10 h at 12°C (Samuelsen and Lunestad, 1996). Muscle and plasma data indicate significant species differences occur in elimination of FLU (in addition to other influences), and withdrawal periods should be established accordingly (Van der Heijden et al., 1994).
Renal excretion cumulatively accounted for 5% of the oral dose of radioactivity in catfish. In bile, 4% of the oral dose was found at 72 h ($t_{\text{max}}$). Bile was not continuously collected and amounts of drug found in the gall bladder probably underestimates total drug excreted by this route, while enterohepatic circulation of FLU is also possible. FLU may be eliminated by other routes (e.g., branchial). Evidence of branchial absorption of FLU was provided by bath exposure studies in freshwater fish, in which significant levels of FLU were found in the tissues after treatment (O’Grady et al., 1988; Hiney et al., 1995). Boon et al. (1991) suggested gill surface area as a possible limiting factor in the elimination of FLU in eel. However, the contribution of the gills in absorption and elimination of FLU remains undetermined.

We identified a taurine conjugate of FLU in bile and urine of catfish and hydroxy-FLU in urine. We did not find metabolites in plasma or muscle tissues. In mammalian species, FLU glucuronide and 7-hydroxy-FLU are principal urinary metabolites (Harri-son et al., 1986; Mevius et al., 1990; Vree et al., 1992). Possibly, FLU glucuronide was unstable in urine and tissues during processing under our conditions; conversely, bile was directly analyzed with no evidence of the glucuronide. Previous studies in fish, while not examining excretory fluids, reported only trace levels of metabolites in tissues (Haagsma et al., 1993; Van der Heijden et al., 1994; Samuelsen and Ervik, 1997).

In summary, FLU was moderately absorbed by the oral route (bioavailability, 44%) and residues were evenly distributed and eliminated in tissues of channel catfish. Parent FLU was the only residue found in edible flesh and was eliminated with a half-life of 26 h. Parent FLU is an appropriate target analyte for residue monitoring programs, for which rapid analytical methods are available. Current data suggest that estimation of withdrawal periods following FLU administration in fish requires individual study for each species under defined environmental and dosage conditions.

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