Pharmacokinetics of oxolinic acid in sea-bass, *Dicentrarchus labrax* (L., 1758), after a single rapid intravascular injection

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The pharmacokinetics of oxolinic acid was studied in sea-bass (*Dicentrarchus labrax*). The fish were kept in seawater at 15.2°C with a 12 h/12 h photoperiod. Oxolinic acid was injected in the caudal vein of anaesthetized sea-bass in a single rapid intravascular administration at a dose of 10 mg/kg of body weight. Plasma concentrations of oxolinic acid were determined using two analytical methods, a classic plate diffusion bioassay using *Escherichia coli* and a high performance liquid chromatography (HPLC) using solid phase extraction with an internal standard and a U.V. detection. The mean recoveries were 99.6% and 110.8% and determination limits were 0.04 μg/mL and 0.02 μg/mL, for the bioassay and the HPLC respectively. Compared to other fish species, the oxolinic acid was rapidly (absorption half life, $t_{a1/2}$ = 0.69 h) distributed to body tissues outside the blood volume (volume of central compartment, V_c = 0.4 L/kg) and presented a large volume of distribution (V_{dss} = 2.55 L/kg). Considering its disappearance from the central compartment (rate constant: central-eliminated, k_{10} = 0.16 h^{-1}) and its total body clearance (Cl_t = 0.066 L/kg.h), the elimination phase of the oxolinic acid in sea-bass was shorter than in trout kept in freshwater, and longer than in salmon in seawater. Consequently, the area under the concentration–time curve (*AUC* = 157 μg.h/mL) and the mean residence time (*MRT* = 42 h) were relatively low and short, respectively.

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INTRODUCTION

In recent years, the 4-quinolone antimicrobial compounds, oxolinic acid and flumequine, have been the most commonly used in commercial aquaculture. Several new fluoroquinolones, including sarafloxacin and enrofloxacin, have been identified as potentially useful therapeutic agents against bacterial diseases of fish (Martinsen *et al.*, 1992; Stamm, 1992; Martinsen & Horsberg, 1995). Despite the wide range of drugs suggested in the scientific literature as being suitable for use in aquaculture, the range legally available to fish farmers is still very limited. Oxolinic acid is officially licensed and widely used in France and many countries (Schlotfeldt, 1992). This first generation 4-quinolone shows a broad-spectrum of activity, particularly against Gram-negative bacteria, in part by interfering with DNA-gyrase (Tsoumas *et al.*, 1989). Consequently, oxolinic acid is particulary efficient against devastating diseases such as Yersiniosis and Vibriosis (Rodgers & Austin, 1983; Gogny *et al.*, 1990). Many studies on oxolinic acid residues have been published on a variety of fish (Kasuga *et al.*, 1984; Archimbault *et al.*, 1988; Ueno *et al.*, 1988a; Ueno *et al.*, 1988b; Jacobsen, 1989; Ishida, 1990; Steffenak *et al.*, 1991; Ishida, 1992). Other studies are available on the pharmacokinetics of oxolinic acid after a bolus intravascular injection, mostly in salmonid fish (Björklund & Bylund, 1991; Hustvedt & Salte, 1991; Hustvedt *et al.*, 1991; Rogstad *et al.*, 1993; Martinsen & Horsberg, 1995). Oxolinic acid is currently used by Mediterranean and Atlantic producers of sea-bass. Nevertheless, no previous report has been published on a pharmacokinetic study on sea-bass (*Dicentrarchus labrax*).

The influence of a variety of factors on pharmacokinetics in fish, including species and environmental parameters (Ingebrigtsen, 1991; Blanc, 1994), require investigation in commercial fish farming. The influence of the species on the pharmacokinetics of oxolinic acid after an intravascular injection has been studied by Jarboe & Kleinow (1990) and Kleinow *et al.* (1994). The influence of water temperature (Endo & Onozawa, 1987a; Björklund *et al.*, 1992; Kleinow *et al.*, 1994), salinity (Endo & Onozawa, 1987b; Ishida, 1992), and also water pH (Endo & Onozawa, 1987a), have been demonstrated.

Among different methods used for determining oxolinic acid in fish tissues, high performance liquid chromatography (HPLC) has been frequently reported (Hustvedt & Salte, 1989; Rasmussen *et al.*, 1989; Rogstad *et al.*, 1989; Björklund, 1990; Samuelsen, 1990). HPLC was chosen for its sensitivity and specificity compared to bioassay but the presence of anaesthetic in blood can make this procedure less suitable (Loussouarn, unpublished data). Bioassay has been less well utilized than the HPLC analytical methods. Nevertheless, bioassay can be used as a reference method for the detection of antimicrobial residues (Froger, 1993) and some works have been recently published using bioassays (Pearson & Inglis, 1993; Barker, 1994).

The primary purpose of the present study was to describe and compare an HPLC technique, using a solid-phase extraction and a U.V. detector, with a classic plate diffusion bioassay using *Escherichia coli*. The second purpose was to study the pharmacokinetics of oxolinic acid administered intravascularly in sea-bass held in defined mariculture conditions.

MATERIALS AND METHODS

Experimental conditions

Experimental fish. Healthy, nonsexed sea-bass (*Dicentrarchus labrax*), weighing 100 ± 10 g, were kept together in a 2 m^3 tank with a continuous seawater flow rate of 0.6 m^3/h. The fish were fed daily with pelleted dry feed. The seawater temperature was maintained at 15.2 ± 0.2°C with an oxygen saturation level kept at 97 ± 1%. The pH and salinity were 7.41 ± 0.07 and 34 ± 0.5%, respectively. The photoperiod was adjusted to 12 h light and 12 h dark. The fish were allowed to adapt to these conditions for at least 2 weeks before the experiment started. They were starved 1 day before drug administration. The experiments were conducted at IFREMER, Noirmoutier Laboratory (France).

Drug administration and sampling. The solution was made by dissolving pure oxolinic acid (Sigma Chemical Co., St. Louis, MO, USA) in 0.03 M NaOH prepared in sterile 9% NaCl. The oxolinic acid concentration in the solution was 5 mg/mL.

For each pharmacokinetic point, fish were randomly selected and anaesthetized individually in aerated seawater containing 0.3 mL/L solution of 2-phenoxyethanol. The fish were individually marked (Arrignon, 1991) and placed on an operating table in order to receive a single rapid injection of 10 mg oxolinic acid per kg of body weight in the caudal vein after dosage adjustment.

The sterile aqueous solution of oxolinic acid was administered by drawing off blood into a syringe (CliniPak 20, Pharma-Plast, Denmark) prior to injection, and then ensuring that the syringe with the drug solution was located in the caudal vein. Otherwise, the fish was excluded and replaced.

After anaesthesia, $\approx$ 1 mL of blood was collected from the caudal vein with heparinized syringes (Tuberculin, Henke, Sass Wolf, Germany), equipped with 0.4×13 mm needles. According to previous studies (Björklund & Bylund, 1991; Hustvedt & Salte, 1991; Hustvedt *et al.*, 1991; Rogstad *et al.*, 1993) the blood samples were taken at 10 and 30 min, 1, 2, 3, 6, 11, 24, 48, 72, 90, 120 and 240 h after drug administration. The 'single fish – single sample' method was used (Horsberg, 1994) and the sampling times were recorded individually. The plasma was isolated by blood centrifugation at 500 ***g*** for 10 min. The samples were immediately frozen and stored at -20°C until analysed.

ANALYTICAL METHODS

Microbioassay

Operating conditions. The classic plate diffusion bioassay used *Escherichia coli* 14 (ATCC 11303) on the agar pH = 6 with a bacterial suspension at 10^5 CFU/mL agar as described by Ellerbroek (1993).

Ninety mL of agar with bacteria added were placed in a sterilized plate (23×23mm) to give a depth of 1.7 mm. When set and cooled, 36 evenly spaced wells, 6 mm in diameter, were made in the agar; 45 µL of solution or plasma could be placed in these wells.

Preparation of standard solutions and plasma samples. For the oxolinic acid solution, the drug was first dissolved in distilled water with NaOH (0.03 N) to give a nominal concentration of 1000 µg/mL. From this solution, further serial twofold dilutions were made in Sörensen phosphate buffer (pH = 8.0) to a final concentration of 0.25–32 µg/mL.

The plasma samples (45 µL) were directly placed in wells after thawing.

Preliminary experiments. The samples of antibiotic-free sea-bass plasma were homogenized in an equal volume of appropriate drug dilutions to obtain the same final dilutions (0.25–32 µg/mL). They were compared to standard solutions in buffer. This bioassay was tested with eight calibrators regularly spaced (0.25–32 µg/mL) on five plates for 4 days.

Determination of oxolinic acid plasma concentrations. Each sample was set in duplicate on each of five plates. Following overnight incubation (18 h) at 37°C, inhibition zones were measured. For each sample, the concentration was obtained by a linear relationship: $Y = aX + b$ (Y = inhibition diameter, X = decimal logarithm of concentration), using the mean of the two inhibition diameters measured on each plate. Then the mean of the five replicate concentrations was retained for pharmacokinetic analysis.

HPLC analysis

A reverse-phase high performance liquid chromatography assay (with an internal standard), using a solid phase extraction procedure and a U.V. detector operated at 340 nm, was developed for the determination of oxolinic acid in anaesthetized sea-bass plasma samples.

Chromatographic system and chemicals. An HPLC system was used, consisting of an L-6200 A solvent-delivery pump and a AS-2000 autosampler (Merck, Hitachi, Dormstadt, Germany), equipped with a L-4250 UV detector (Merck) and a Deskpro 386S/20 computer (Compaq, Houston, TX, USA). Peak heights, areas and concentrations were calculated with a D-6000 HPLC-Manager software (Merck). The LichroCart column (125 × 4mm) and the guard column (4 × 4 mm) were packed with 5 μm particle diameter LiChroSpher 100RP-18 End Capped phase (Merck, Dormstadt, Germany). All reagents used were of analytical or HPLC grade.

Preparation of standard solutions. Stock solutions of oxolinic acid and nalidixic acid (internal standard) were prepared at 1000 μg/mL in 0.03 M aqueous NaOH solution. Working standards were prepared by dilution in Sörensen phosphate buffer (0.2, 1, 5 and 25 μg/mL) and spiked with internal standard (10 μg/mL).

Sample preparation. One hundred μL of defibrinated plasma sample were spiked with internal standard (20 μg/mL for a final concentration of 10 μg/mL), shaken and cleaned by solid-phase extraction procedure with Bond Elut® column C18 (Varian, USA) (1 mL). The extraction procedure was implemented according to Rasmussen *et al.* (1989) and Pouliquen *et al.* (1994), slightly modified. Oxolinic acid and nalidixic acid were eluted with six 250 μL volumes of acetonitrile. The organic phase was collected and evaporated to dryness under nitrogen at 40°C. Prior to analysis, the dry residues were reconstituted with 200 μL of phosphate buffer.

The levels of oxolinic acid were determined by reverse-phase high-performance liquid chromatography using a C18 End-Capped column and a U.V. detector operated at 340 nm. The mobile phase was 0.02 M orthophosphoric acid-acetonitrile-dimethylformamide (60: 10: 30, v/v) with a flow rate of 0.8 mL/min. The sample size injected was 50 μL.

Preliminary experiments. The samples of antibiotic-free sea-bass plasma were homogenized in an equal volume of appropriate drug dilutions to obtain the same final dilutions (0.20–25 μg/mL) with internal standard. Known samples were cleaned as described above. Each sample was injected three times per day for 3 days. This analytical method was tested for 3 days on a 4-point concentration range.

Determination of oxolinic acid plasma concentrations. The oxolinic acid concentrations were calculated by the software described above with a standard curve. Each day a calibration curve was obtained from antibiotic standards prepared in phosphate buffer. At the same time, sea-bass plasma samples spiked with oxolinic acid were prepared to check the calibration curve.

Validation assay methodology

The validation of both methods was performed using spiked sea-bass plasma samples. The specificity was demonstrated after the analysis of drug-free plasma and plasma spiked with standard mixed solutions, to check for any compounds interfering with oxolinic acid and nalidixic acid, and to identify their retention times. Precision (repeatability and reproducibility) was assessed by determining the within and between run variability according to an analytical validation guide (Caporal-Gautier & Nivet, 1992). The recoveries of oxolinic acid were determined by comparing spiked blank plasma with standard solutions for each calibration. The independency between coefficients of variation and concentrations was also checked. The linearity, using ordinary and weighted least squares, was tested by ANOVA. The quantification limit was defined as the lowest concentration of oxolinic acid in plasma with a predefined level of confidence (Liteanu & Rica, 1980). A coefficient of variation of 20% is commonly used to define the determination level. Determination limits were estimated from ordinary or weighted least squares regressions with specific software developed in our laboratory according to Aarons *et al.* (1987).

PHARMACOKINETIC ANALYSIS

All data sets were analysed for the best fit to pharmacokinetic models by least-squares nonlinear regression analysis. In the present study, the *F* ratio test (Yamaoka *et al.*, 1978a) was used to determine the best structural model.

The diffusion and elimination processes were assumed to follow first order kinetics and pharmacokinetic parameters were determined according to Gibaldi & Perrier (1982).

Area under the plasma drug concentration vs. time curve from zero to infinity (AUC_0-∞) and area under first moment curve ($AUMC_0$-∞) were calculated using the linear trapezoidal rule directly on the observed concentrations. The mean residence time (*MRT*) of a drug in the body was calculated according to Yamaoka *et al.* (1978b). The total body clearance (Cl_t) and the apparent volume of distribution at steady state (V_{dss}) were determined either with compartmental or noncompartmental approaches.

RESULTS

Analytical method validation

The method for validating the use of HPLC and bioassay was verified using the concentration and the inhibition diameter, respectively. Results from validation are shown in Tables 1 and 2.

Mean-recoveries obtained were 110.8% and 99.6% for the HPLC and bioassay, respectively. The recovery for both analytical methods tested by ANOVA was found to be not concentration-dependent (Fig. 1a & b). Consequently, the variance increases in proportion to the concentration squared and therefore weighted least-squares could be used to analyse the data (Garden *et al.*, 1980).

On one hand, because of the small number of calibrators, the weighted least-squares (MCW) was used for HPLC data analysis

Table 1. Precision (repeatability and reproducibility), recovery, linearity and quantification limit (μg/mL) of oxolinic acid by microbioassay

	Bioassay							
n	20	20	20	20	20	20	20	20
Dilution (μg/mL)	0.25	0.5	1	2	4	8	16	32
CVR (%)	8.79	8.55	5.24	3.97	2.65	3.10	3.07	3.56
Recovery ± SD (%)	111.3 ± 4.2	94.1 ± 5.6	99.3 ± 5.4	86.3 ± 6.0	111.5 ± 6.8	95.8 ± 3.5	102.4 ± 6.5	96.1 ± 7.8
Calibration curve	$Y = 11.15 \times C + 18.49$							
R^2	0.963							
F ratio	8400.63							
F 0.05 (1, 159)	3.91							
Determination limit (μg/mL)	0.044 (MCO)							

n, data number; CVR, reproducibility coefficient of variation; SD, standard deviation; R^2, coefficient of linear regression; *F* ratio, test of Fisher Snedecor, *F* 0.05 (1, ddl), table reference at the critical value of 5%; MCO, ordinary least squares.

Table 2. Precision, recovery, linearity and quantification limit (μg/mL) of oxolinic acid by HPLC

	HPLC			
n	8	9	9	9
Dilution (μg/mL)	0.2	1	5	25
CVR (%)	1.87	0.33	0.14	0.16
Recovery ± SD (%)	117.3 ± 26.2	111.4 ± 8.6	109.8 ± 9.5	104.7 ± 6.6
Calibration curve	$C = ((R - 1.46)/1.05) + 1.34$			
R^2	0.991			
F ratio	3554.73			
F 0.05 (1, 34)	4.14			
Determination limit (μg/mL)	0.020 (MCW)			

n, data number; CVR, reproducibility coefficient of variation; SD, standard deviation; R^2, coefficient of linear regression; *F* ratio, test of Fisher Snedecor; *F* 0.05 (1, ddl), table reference at the critical value of 5%; MCW, weighted least squares.

(Aarons *et al.*, 1987). On the other hand, the ordinary least-squares were performed in the bioassay for which data were more numerous.

All relative standard deviations for between-day precision were less than 10% for bioassay (see Table 1), and less than 3% for HPLC (see Table 2).

For each analytical method, the linearity was accepted with the *F* ratio test (see Tables 1 & 2). Variation coefficients of the predicted concentrations were calculated and are displayed in Fig. 2. The corresponding quantification limits were, respectively, evaluated at 0.0195 μg/mL and 0.044 μg/mL for HPLC and bioassay (see Aarons *et al.*, 1987).

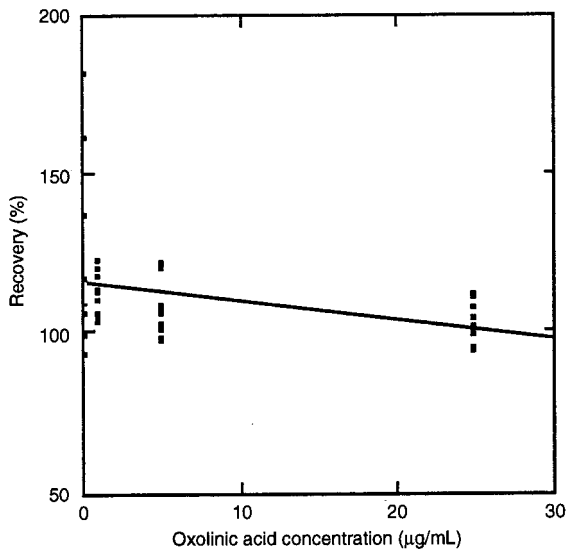

Fig. 1a. Distribution of HPLC recoveries vs. concentrations.

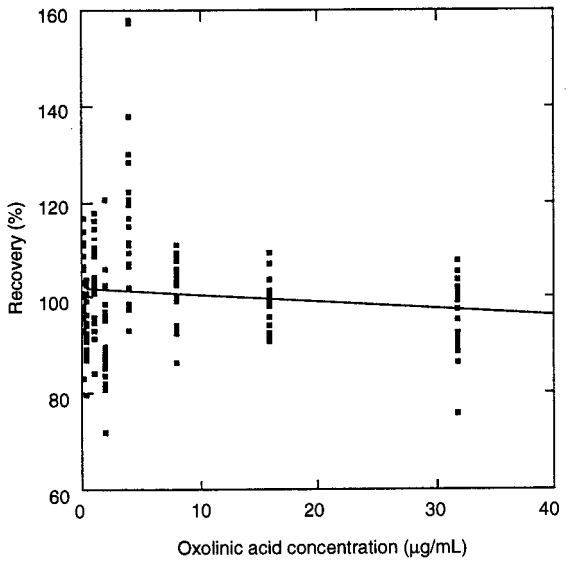

Fig. 1b. Distribution of bioassay recoveries vs. concentrations.

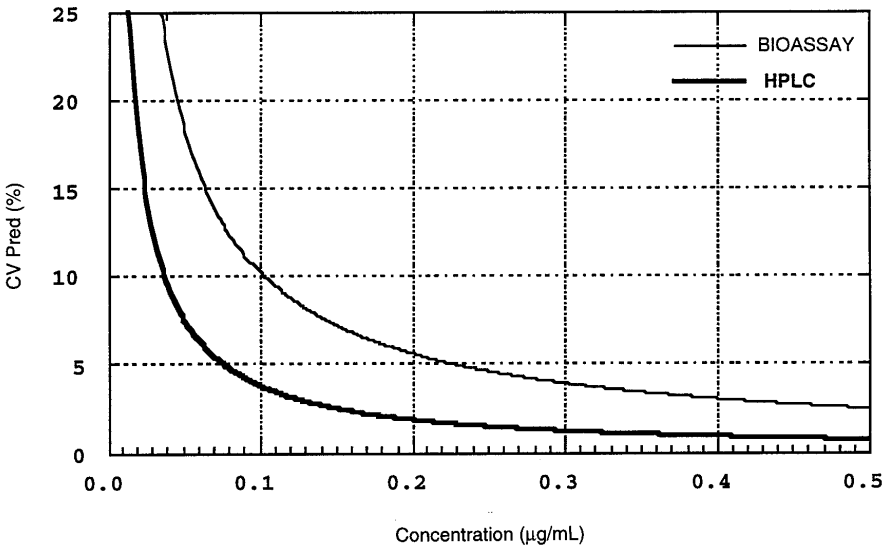

Fig. 2. Coefficient of variation of the predicted concentrations (CV Pred) after bioassay and HPLC methods.

Plasma concentrations

The mean plasma drug concentrations measured after a single intravascular injection of oxolinic acid in sea-bass are listed in Table 3. The data reported in this study were not corrected for recovery. The data number per sampling time was variable (Table 3) because of experimental or sample preparation difficulties. The concentrations obtained by both analytical methods were compared by a Wilcoxon-Mann–Whitney test (Scherrer, 1984). For each time period, the concentrations were not statistically different at a 5% significance level (Table 3). Consequently, the plasma concentration data of both analytical methods were kept for the calculation of the mean concentrations (Table 3).

Pharmacokinetics

The data resulting from intravenous dose administration were initially subjected to graphic analysis by a semilogarithm plot of log plasma concentration as compared to time (Fig. 3). The residual method was used to define the initial estimates of the hybrid rate constants as well as the Y-intercepts (Shargel & Yu, 1985). The *F* ratio tests (Table 4) indicate that the data were best described with a three-compartment open model using three-exponential curve fitting:

$$Cp(t) = A \cdot e^{-a \cdot t} + B \cdot e^{-b \cdot t} + C \cdot e^{-c \cdot t},$$

where $Cp(t)$ is the plasma concentration, t is the time, a,b and c are slopes of mono-exponential declining curves ($a > b > c$), and A, B and C are the zero-time plasma concentrations. A model with input into, and elimination from, the central compartment, was retained.

The predicted concentrations were semilogarithmically plotted against time with the observed concentrations (Fig. 3), and the pharmacokinetic values calculated from this model are shown in Table 5.

The plasma drug concentration–time profile showed three

Table 3. Mean oxolinic acid concentration (µg/mL) in plasma after a single rapid intravascular injection of 10 mg/kg in sea-bass at 15°C, determined using HPLC and microbioassay

	Concentration (µg/mL ± SD)				
Time (h)	HPLC	Bioassay	U	Mean concentration	*n*
0.167	24.38 ± 7.05	19.04 ± 4.43	5*	23.41 ± 6.81	11
0.5	14.07 ± 3.24	16.22 ± 1.33	8*	14.56 ± 3.01	13
1	11.96 ± 2.72	11.33 ± 2.20	12*	11.81 ± 2.52	12
1.75	7.51 ± 1.36	6.96 ± 1.18	10*	7.38 ± 1.30	13
3	5.54 ± 1.19	5.70 ± 2.46	11*	5.59 ± 1.48	11
6	4.03 ± 1.29	2.41 ± 1.62	6*	3.62 ± 1.49	12
11	2.47 ± 0.61	2.45 ± 0.19	9*	2.46 ± 0.51	10
24	1.86 ± 0.91	1.42 ± 0.19	6*	1.73 ± 0.78	10
48	0.74 ± 0.47	0.60 ± 0.30	10*	0.70 ± 0.42	11
72	0.58 ± 0.13	0.30 ± 0.13	1*	0.46 ± 0.19	7
90	0.22 ± 0.12	0.27 ± 0.08	5*	0.24 ± 0.10	8
120	0.10 ± 0.08	n.d.		0.10 ± 0.08	6
240	0.04 ± 0.03	n.d.		0.04 ± 0.03	2

n.d., not determined; SD, standard deviation; *n*, data number; *U*, Wilcoxon-Mann-Whitney test; *differences are not significant at the level of 5%.

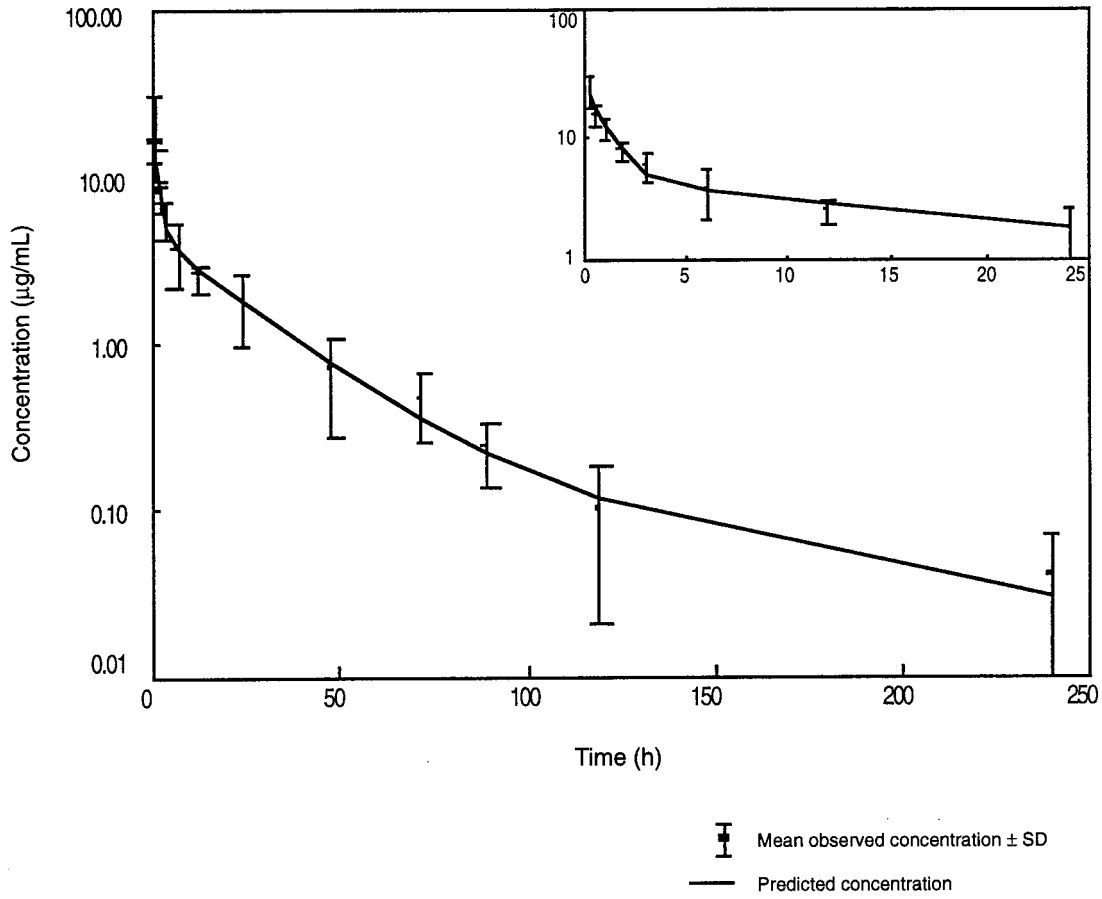

Fig. 3. Semi-logarithmic plot of mean (± SD) plasma concentration vs. time curves and predicted concentrations in sea-bass after a single rapid intravascular injection of oxolinic acid (10 mg/kg).

Table 4. Results of residuals sum-squared (SCER) and the *F* ratio test with ddl for the compartment open models

	SCER	ddl	*F* ratio
1 compartment open model	58.54	2,124	
2 compartment open model	27.03	2,122	71.11*
3 compartment open model	19.94	2,120	21.33*

ddl = (2, dfN) with dfN = data number $-2 \times$ compartimental number.
*Non significant value at the significant level of 5%

distinct phases. The rapid initial decline of plasma oxolinic acid concentration was mainly the result of drug distribution from the highly perfused central compartment to a tissue compartment of sea-bass ($k_{12} = 0.66\ h^{-1}$), and to the third or deep tissue compartment ($k_{13} = 0.02\ h^{-1}$). The central compartment volume (V_c) was large, 0.4 L/kg (more than 7 times the blood volume (Stoskopf, 1993)).

The apparent distribution volume (V_{dss}) was 2.55 L/kg and the a-phase half-life ($t_{a1/2}$), dominated by the distribution process, was 0.69 h. The c-phase half-life ($t_{c1/2}$), representing the elimination of the drug from the body, was longer (87 h) than the elimination from the central compartment ($t_{1/2} = \ln 2/k10 = 4.25$ h). Consequently, during the terminal phase, the rate of the elimination process was limited by back distribution from the peripheral compartments. The total body clearance (Cl_t) reached 0.066 L/kg.h (1.584 L/kg.24 h). Therefore, the area under the curve (AUC_0-∞) was 157.25 µg/mL.h, and the mean residence time (*MRT*) was 42.27 h.

DISCUSSION

Many disadvantages associated with the use of bioassay, thought to cause a lack of precision and sensitivity, have been expressed by McGill & Hardy (1992). Interference of anaesthetic on analysis of oxolinic acid using an HPLC method has not been previously reported. This interference led to the modification of HPLC operating conditions, such as the wave-length, therefore reducing the sensitivity of the HPLC analytical method. With the HPLC technique, the oxolinic acid concentration levels were detected up to 240 h; but owing to the lower sensitivity of the bioassay, the concentrations were detected only up to 90 h. Microbiological assays, although usually not as sensitive as HPLC techniques, are easier to perform (the samples were directly placed in wells without extraction) and require fewer resources. Moreover, in this study, similar precision, recovery and linearity were achieved with the bioassay. The sensitivity of bioassay could be improved by a simple concentration of the low-

Table 5. Pharmacokinetic parameters of oxolinic acid in sea-bass after a single rapid intravascular injection of 10 mg/kg with abbreviations

Parameter	
Seawater temperature (°C)	15.2 ± 0.2
Fish weight (g)	100 ± 10
Number of fish	2–13
Dose (mg/kg)	10
Compartmental model	
A (μg/mL)	20.392
B (μg/mL)	4.163
C (μg/mL)	0.200
a (h^{-1})	1.009
b (h^{-1})	0.039
c (h^{-1})	0.008
$C_p(0)$ (μg/mL)	24.75
$t_{a1/2}$ (h)	0.69
$t_{b1/2}$ (h)	17.77
$t_{c1/2}$ (h)	86.64
k_{10} (h^{-1})	0.163
k_{12} (h^{-1})	0.655
k_{13} (h^{-1})	0.020
k_{21} (h^{-1})	0.209
k_{31} (h^{-1})	0.009
V_c (L/kg)	0.40
V_{dss} (L/kg)	2.55
Cl_t (L/kg.h)	0.066
Non compartmental model	
AUC_0-∞ (μg/mL.h)	157.25
AUMC (μg/mL.h^2)	6646.52
MRT (h)	42.27
*V_{dss} (L/kg)	2.69
*Cl_t (L/kg.h)	0.064

level samples. Consequently, bioassay could be successfully used to quantify one drug for a pharmacokinetic study.

The large apparent volume of the central compartment (V_c = 0.40 L/kg) and the low half-life of the a-phase dominated by the distribution process ($t_{a1/2}$ = 0.69 h) in sea-bass indicated that oxolinic acid was initially distributed rapidly to tissues outside the blood volume of fish. Björklund & Bylund (1991) have shown the same V_c in rainbow trout (*Oncorhynchus mykiss*) kept in freshwater at the same temperature and oxolinic acid dosage (V_c = 0.399 L/kg). The volumes in the central compartment were relatively similar whatever the environment or species.

The constants for the drug distribution between the central to peripheral compartments (k_{12} = 0.66 h^{-1} and k_{13} = 0.02 h^{-1}) were lower in sea-bass than the values previously reported in trout. At the same dose in rainbow trout, Hustvedt & Salte (1991) estimated the k_{12} and k_{13} to be 4.26 h^{-1} and 0.56 h^{-1}, respectively, in seawater at 8.5°C, and Björklund & Bylund (1991) estimated a k_{12} of 1.82 h^{-1} in freshwater at 16°C calculated with a two-compartmental open model. However, in sea-bass, back distribution of oxolinic acid from the peripheral compartments to blood circulation (k_{21} = 0.209 h^{-1} and k_{31} = 0.009 h^{-1}) was shorter than in rainbow trout in seawater at 8.5°C (k_{21} = 1.71 h^{-1} and k_{31} = 0.07 h^{-1}) and in freshwater (k_{21} = 0.44 h^{-1}). For both species, sea-bass and rainbow trout kept in freshwater (Björklund & Bylund, 1991), the *V*c and the initial concentration *C*p(o), 24.75 μg/mL and 29.9 μg/mL, respectively, were similar. It should be emphasized that the difference between k_{12} and k_{21} was more than three times higher in trout than in sea-bass. Consequently, the distribution process was faster in trout than in sea-bass.

The apparent volume of distribution at steady state (V_{dss}), because of its nonmodel-dependency, would likely be better for comparisons than the apparent volume based on area under the curve (V_{darea}) which may be overestimated by the model slope β. Compared to sea-bass, the V_{dss} for the trout kept in freshwater was lower (1.9 L/kg: Björklund & Bylund, 1991), and for the Atlantic salmon (*Salmo salar*) in seawater, it was higher (5.4 L/kg: Martinsen & Horsberg, 1995; 3.876 L/kg: Rogstad *et al.*, 1993). Therefore, the V_{dss} is species-dependent and seems to be larger in seawater species.

In seawater species, the disappearance of oxolinic acid from the central compartment (k_{10}) was 0.163 h^{-1} and 0.36 h^{-1} for sea-bass and trout (Hustvedt & Salte, 1991), respectively. In freshwater species, k_{10} for the trout was lower than 0.06 h^{-1} (Björklund & Bylund, 1991). The elimination half-life ($t_{c1/2}$ = 86.6 h) of oxolinic acid in sea-bass was longer than in rainbow trout kept in seawater at 8.5°C ($t_{c1/2}$ = 29.1 h) and in freshwater at 16°C ($t_{b1/2}$ = 69.7 h). In the same way, the elimination half-life for the salmon in seawater at 9°C with a dose of 20 mg of oxolinic acid per kg ($t_{c1/2}$ = 60.3 h: Hustvedt *et al.*, 1991) was shorter than in sea-bass.

The total body clearance (Cl_t) in sea-bass was calculated to be 0.066 L/kg.h. It exceeds the Cl_t reported for the rainbow trout kept in freshwater (0.02 L/kg.h: Björklund & Bylund, 1991). However, it is much lower than the Cl_t observed for the salmon kept in seawater (0.20 L/kg.h: Rogstad *et al.*, 1993; 0.28 L/kg.h: Martinsen & Horsberg, 1995). Consequently, the area under the curve (AUC_0-∞) was lower for the salmon (24 μg.h/mL: Rogstad *et al.*, 1993; 89 μg.h/mL: Martinsen & Horsberg, 1995) than for the trout kept in freshwater (453 μg.h/mL: Björklund & Bylund, 1991). The sea-bass, with an *AUC* of 157 μg.h/mL and an *MRT* of 42 h, reacted similarly to trout in seawater at 8.5°C (AUC_0-∞ = 200 μg.h/mL and *MRT* = 37 h) reported by Hustvedt & Salte (1991).

In addition, the elimination phase has been shown to be faster in seawater than in freshwater fish.

For the Atlantic salmon, Rogstad *et al.* (1993) and Martinsen & Horsberg (1995) have reported similar values for the *Clt*, *AUC* and *MRT*, which were larger than those calculated by Hustvedt *et al.* (1991). These comparatively large discrepancies could be explained by the fish cannulation which altered pharmacokinetic properties compared to those of noncannulated fish as suggested by Horsberg (1994).

The data previously published (Tsoumas *et al.*, 1989; Lewin & Hastings, 1990; Martinsen *et al.*, 1992), concerning the minimum inhibitory concentration (*MIC*) of oxolinic acid *in vitro* for many susceptible strains (*Aeromonas salmonicida*, *Vibrio anguilarum*, *Vibrio salmonicida* and *Yersinia ruckeri*), ranged from 0.005–0.5 μg/mL (for most strains, < 0.1 μg/mL). Thus, an

oxolinic acid bolus of 10 mg/kg in sea-bass kept in seawater at 15°C, should maintain the concentration above this *MIC* value for 2 days or more than 4 days. Moreover, the high partition of oxolinic acid in the octanol phase (Björklund & Bylund, 1991) explains the large distribution in skin and muscle, which are only slightly vascularised in fish (Ellis *et al*., 1989), and where abcesses caused by bacteria may occur.

In conclusion, this first report concerning pharmacokinetics of oxolinic acid in sea-bass emphasizes that: (1) the distribution process of oxolinic acid is faster in trout; and (2) the elimination phase is shorter in seawater than in freshwater fish.

Obtained results suggest a good efficacy when this antibacterial agent is administrated by a bolus injection possibly used in sire stock. The distribution of the oxolinic acid in sea-bass, and its elimination processes were lower than in salmon, demonstrating that this drug warrants further analysis for sea-bass therapeutics. Nevertheless, in brood stock, oxolinic acid is usually administered orally. Therefore, for its large scale therapeutic application, we will need to do further studies to determine the species-dependent bioavailability of this drug. It is only then that we will be able to confirm the effectiveness of oxolinic acid in sea-bass.

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ABBREVIATIONS

A, B, C	Zero plasma drug concentration intercepts of intravenous disposition curve.
a, b. c	Slopes of distribution and terminal phases, respectively, of three-exponential drug disposition curve after an intravascular dose.
$C_p(0)$	Plasma drug concentration immediately after bolus injection
$t_{a,b,c½}$	Distribution and elimination half-lives.
k_{10}	First-order rate constant for disappearance of drug from the central compartment.
$k_{12,21,13,31}$	First-order rate constant for drug distribution between the central and peripheral compartments.
V_c	Apparent volume of the central compartment. V_c = Dose IV/C_p.
V_{dss}	Apparent volume of drug distribution at steady state. $V_{dss} = V_c + k_{12}/k_{21} \times V_c + k_{13}/k_{31} \times V_c$.
$^*V_{dss}$	$^*V_{dss} = MRT \times Cl_t$.
Cl_t	Total body clearance, which represents the sum of all clearance processes in the body. $Cl_t = V_c \times k_{10}$.
$^*Cl_t$	$^*Cl_t$ = dose/AUC.
AUC_0-∞	Area under the plasma drug concentration–time from zero to infinity.
$AUMC_0$-∞	Area under the curve of a plot of the product of time and plasma drug concentration–time.
MRT	Mean residence time. $MRT = AUC/AUMC$, which represents the statistical mean of the time that the drug molecule resides in the body.

*Determined by noncompartmental approach.