OXOLINIC ACID

First draft prepared by
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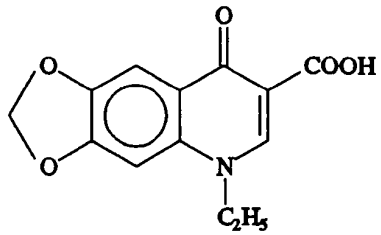
IDENTITY

Chemical names: 5-ethyl-5,8-dihydro-8-oxo-1,3-dioxolo[4,5-g]quinoline-7-carboxylicacid; 1-ethyl-1,4-dihydro-6,7-methylenedioxy-4-quinolone-3-carboxylic acid

Synonyms: W 4565, Emyrenil, Nidantin, Ossian, Ozoboi, Pietil, Prodoxol, Urinox, Uritrate, Uro-Alvar, Urotrate, Uroxin Von Boch, Uroxol, Utibid.

Structural formula:



Molecular formula: $C_{13}H_{11}NO_5$

Molecular weight: 261.24

OTHER INFORMATION ON IDENTITY AND PROPERTIES

Pure active ingredient: Oxolinic acid

Appearance: Colourless powder or crystals

Melting point: 314-316°C

Optical rotation: Optically inactive

Mode of Action: Detailed studies were made of the effect of oxolinic acid on the growth of *Proteus vulqaris*. Using appropriate culture media, the organism was grown in the presence of a number of isotopically labelled precursors of cell constituents. These included thymidine, uracil and L-valine incorporation (Parke-Davis, 1994).

It was found that exposure of growing cells to various concentrations of oxolinic acid markedly inhibited DNA synthesis in a dose-related manner. Protein synthesis was also suppressed, but to a lesser extent. Oxolinic acid had no effect on the synthesis of RNA. Furthermore, the cell wall was not altered by oxolinic acid as shown by no leakage of d-glucose and no depression of cell wall lipid.

Indications: The major use of the substance is in aquaculture, both as a therapeutic and prophylactic agent in fish.

Oxolinic acid is a synthetic antibacterial agent used in many countries in the treatment of fish diseases. The compound has been used successfully for the control of furunculosis (Endo et al., 1973; Austin et al., 1983), vibriosis

(Endo et al., 1973; Austin et al., 1982) and enteric redmouth disease (Rodgers and Austin, 1983). Oxolinic acid is administered to fish mixed in the feed usually for 10 days at doses of 10-30 mg/kg body weight.

Fish: Furunculosis, yersiniosis, vibriosis, cold-water vibriosis

RESIDUES IN FOOD AND THEIR EVALUATION

CONDITIONS OF USE

General

Oxolinic acid is a synthetic antimicrobial agent which is similar in structure to the naturally occurring nalidixic acid, but is more active against both Gram-negative and Gram-positive bacteria although it is used almost exclusively for its Gram-negative microbiological activity. Oxolinic acid is an older member of a group of synthetic antimicrobial agents generically termed the *quinolones*. Although more recent additions to the quinolone drug family out-perform oxolinic acid with respect to both bacteriocidal activity and bioavailability, its relatively modest cost, low mammalian and fish toxicity and satisfactory performance render it a useful and widely used drug, particularly in the aquaculture industry. Human oral absorption of oxolinic acid is low. It is excreted in the urine and has been used in human medicine for the treatment of urinary tract infections, although alternative treatments are often preferred.

Oxolinic acid has shown activity against a broad range of Gram-negative organisms, being especially active against proteus species but less active, or inactive, against pseudomonas.

The spectrum of antimicrobial activity of oxolinic acid is similar to that of nalidixic acid except that oxolinic acid is also active against staphylococci. Oxolinic acid is not particularly effective against other pathogenic Gram-positive bacteria.

Oxolinic acid is also used in veterinary medicine for the treatment of disorders arising from Gram-negative infections. However, by the far the major use of the drug is in the aquaculture industry where, in addition to its Gram-negative action, its broad spectrum of activity against fungi, protozoans and helminths have resulted in its effective use in fish farming in many countries, both as a prophylactic as well as a chemotherapeutic agent.

Oxolinic acid has a high activity against *Aeromonas salmonicida.* The Minimum Inhibitory Concentration (MIC) values ranged between 0.0075 - 0.03 μg/mL for 10 isolates tested. These MIC values were markedly lower than the corresponding MIC values for nalidixic acid which ranged between 0.06 - 0.125 μg/mL (Parke-Davis, 1994).

Further experiments again showed the sensitivity of pathogenic strains of *A. salmonicida* to oxolinic acid and also showed the quick inhibiting effect on bacterial cultures of *A. salmonicida* insofar as the bacterial cultures were inhibited within 5 minutes by a drug concentration of 50 mg/L. In field studies on salmon, trout and carp, oxolinic acid was found to be effective in treating *A. salmonicida* infections at doses between 5-20 mg/kg, whereas nalidixic acid was much less effective.

Endo et. al. (1984) also showed that oxolinic acid had a MIC of 0.02 μg/mL against the one strain of *A. salmonicida* tested and also demonstrated the MIC of oxolinic acid against the 7 strains of *A. liquefaciens* tested ranged between 0.02-0.09 μg/mL and against the 5 strains of *Vibrio anquillanium* tested with a range of 0.02 to 0.09 μg/mL.

Dosage

Species:	fish species
Routes of administration:	oral
Dose	10-50 mg/kg

PHARMACOKINETICS

General

Very little if anything, is known about the distribution or the degree of protein binding of oxolinic acid in different tissues and organs (Hustvedt and Salte, 1991). Similarly, there is no information as to the pattern of distribution of oxolinic acid in fish, i.e. whether the drug is homogeneously distributed.

In rainbow trout, there was a significantly lower terminal elimination half-life in seawater than in freshwater. This difference was attributed to the higher total clearance in seawater, i.e. the sum of all elimination processes in the fish. This may be caused by differences in the rate of elimination, metabolism or both. The urine flow rate in salmonids is dramatically reduced in seawater compared to freshwater (Hunn, 1982). Thus, renal excretion of the drug could not be a rate-limiting step in the elimination of oxolinic acid under usual experimental conditions. Approximately 50% of oxolinic acid in the plasma at therapeutic concentrations is unbound (Benjaminsen and Hustvedt, 1986) and can freely interact with the secretory system in the nephrones. The secretion of some xenobiotics is higher in fish compared with mammals (Pritchard and Bend, 1984), this may also be true for oxolinic acid.

Giant Prawn *(Panaeus monodon)*

The pharmacokinetics of oxolinic acid haemolymph clearance, absorption, tissue distribution and excretion were investigated in the giant prawn *(Penaeus monodon)* after single intramuscular injection and oral administration to prawns of 30-40 g body weight at 28-32°C (Limpoka et. al., 1994). Elimination half-life was 4.68 hours following intramuscular injection of 10 mg/kg of oxolinic acid with an apparent volume of distribution of 0.22 L/kg. At a higher dose of 20 mg/kg the distribution phase revealed a half-life of 3.36 h and an elimination half-life of 5.37 hours. No drug levels occurred in tissue above 10 ng/g 5 days after injection. The drug was rapidly absorbed after single oral dosing. The peak haemolymph concentration of 0.45 μg/mL and 0.55 μg/mL ocurred 0.87 and 0.81 hours after forced oral administration, respectively. There was a good correlation between the concentration of the drug found in haemolymph and tissue levels. No drug levels occurred in tissue above 10 ng/g 3 days after the single oral dose.

Prawns were fed for 5 days on a diet containing oxolinic acid at 0.5 g/kg and 1.0 g/kg in the feed respectively. The edible tissues of prawns contained less than 10 ng/g oxolinic acid six days after withdrawal of medicated pelleted feed and 7 days after withdrawal of medicated fish flesh diet. The haemolymph and tissue concentrations of oxolinic acid during the feeding of prawns with medicated fish flesh feed for 5 days followed by withdrawal from the drug is shown in Table 1.

Giant Sea Perch *(Lates calcarifer)*

The clearance of oxolinic acid from plasma and tissue in the Giant Sea Perch *(Lates calcarifer)* after single oral administration and 5 days on medicated diet has been investigated (Chai-anan et. al., 1993). Following a single oral dose of 20 mg/kg, peak plasma and tissue concentrations were found after 1 hour and fell below the limit of detection in 72 hours post-dosing. There was a good correlation between the concentration of the drug found in plasma with levels in edible tissues at each sampling time point (Table 2).

Fish were fed for 5 days on a diet containing oxolinic acid at 1.0 g/kg feed . The plasma contained above 1 μg/g oxolinic acid during medicated feeding. Plasma levels of oxolinic acid fell below the detection limit of 200 ng/kg on day 8 after withdrawal of medicated diet. There was a good correlation between the concentration of the drug found in plasma with levels in edible tissues, the ratio at each sampling time point being 1.1-1.5 : 1. Therefore, for fish maintained at 30°C and fed on a medicated diet of 1 g/kg of oxolinic acid, a drug withdrawal period of 8 days was recommended.

Table 1. Mean Prawn Tissue and Haemolymph Concentrations of Oxolinic Acid Following Adding 1 g and 2 g Aquinox/kg Fish Flesh Feed for 5 Days[1].

Time (hr)	Days	Haemolymph Conc. (μg/mL)[3]		Tissue Conc. (μg/g)	
		Dose 1 g/kg	Dose 2 g/kg	Dose 1 g/kg	Dose 2 g/kg
0	1	0	0	0	0
4		0.82 ± 0.12[2]	1.15 ± 0.18[2]	1.83[2]	2.89[2]
24	2	0.65 ± 0.30	1.00 ± 0.16	1.25	1.70
28		0.75 ± 0.10	1.28 ± 0.10	1.30	5.11
48	3	0.50 ± 0.30	0.75 ± 0.10	1.20	1.80
52		0.85 ± 0.20	0.92 ± 0.07	1.30	2.89
72	4	0.58 ± 0.20	0.97 ± 0.27	0.98	1.82
76		0.93 ± 0.20	1.20 ± 0.50	0.44	3.34
96	5[4]	0.80 ± 0.05	1.0 ± 0.20	1.10	1.67
98		1.0	1.37 ± 0.30	4.40	5.09
100		0.09 ± 0.07	1.20 ± 0.16	1.70	3.22
104		0.87 ± 0.08	1.0	1.60	1.60
120	6	0.60	0.93 ± 0.90	0.70	1.94
144	7	0.25	0.825 ± 0.17	0.50	0.85
168	8	trace	0.25	0.45	0.50
192	9	ND	0.25	0.35	0.40
216	10	ND	trace	0.02	0.20
248	11	ND	trace	ND	0.10
264	12	ND	ND	ND	ND

1. Pharmsure Aquinox = 50% oxolinic acid
2. Mean from four different heamolymph samples, microbiological assay, LOD 250 ng/g.
3. Assay by HPLC, limit of detection = 10 ng/g, mean from pooled samples.
4. The last day of drug dosing

ND = not detected; trace = < 250 ng/g

Table 2. Mean Fish Tissue and Plasma Concentrations of Oxolinic Acid Following a Single Oral Dose of 20 mg/kg

Time (hours)	Oxolinic acid concentration (μg/ml) Plasma[1]	(μg/g) Tissue[1]	Ratio of concentrations Plasma/Tissue
0.3	0.85 ± 0.40	0.80	1.06
1	1.25 ± 0.15	1.05	1.19
2	0.50 ± 0.10	0.40 ± 0.20	1.25
4	0.45 ± 0.20	0.30 ± 0.01	1.50
8	0.25	0.02 ± 0.015	1.25
12	<0.25	0.40 ± 0.01	
24	<0.25	0.30	
30	<0.25	0.02	
48	trace	0.01	
72	n.d.	n.d.	

[1] Mean from three individual fish; n.d.= not detected

Atlantic salmon *(Salmo salar L.)*

Hustvedt (1993a) has reported on the administration of oxolinic acid as a single dose to Atlantic salmon *(Salmo salar L.)*. Twelve fish weighing 923 ± 202 g, maintained between 8.0 and 8.1°C, were given a mean single

oral dose of 25.9 mg oxolinic acid per kg body weight administered as 0.5 % medicated pellets. Blood samples were taken via a cannula at 3, 6, 9, 12, 24, 48, 96, 144 and 192 hours after the administration of oxolinic acid. The serum samples were cleaned up by a solid phase extraction procedure and oxolinic acid concentrations were determined in duplicate by high-performance liquid chromatography as described by Thanh (1988). The analytical method gave recoveries of 82%, 85% and 90%, and the coefficients of variation were 8%, 6% and 5% at 0.01, 1.0 and 5.0 μg/mL, respectively. The detection limit (signal to noise ratio 3:1) was 1 ng/mL.

The bioavailability of oxolinic acid in this study was estimated by using the pharmacokinetic constants following a single intravascular injection of oxolinic acid presented by Hustvedt et. al. (1990b). Thus AUC was estimated to be 782 $g \cdot h \cdot mL^{-1}$ after 20 mg oxolinic acid per kg body weight. The elimination processes were all assumed to follow first order kinetics. All pharmacokinetic values were determined according to Rowland and Tozer (1989). The mean values were estimated based upon mean serum concentrations from all fish (n=9).

The estimated mean bioavailability of oxolinic acid after a single oral dose of 0.5% medicated feed was 19.9%. The mean fraction of available dose remaining in the fish at 192 h was 0.075. Mean T_{max} was 24.3 h (range 17.0-33.3 h), and C_{max} was 2.1 μg/ml (range 1.5-4.0 μg/ml) while the lag time (T_{lag}) was 2.0 h (range 0-4.2 h). Over all mean elimination half-life was 55.4 h while individual fish ranged from 28.4 to 65.2 h. Although the bioavailability of oxolinic acid was low, serum levels which were achieved should be sufficient to obtain satisfactory serum levels after multiple dosing.

The serum profile of oxolinic acid after multiple doses has been studied by Hustvedt (1993b). Fish, maintained at a mean water temperature of 7.8°C and a mean salinity of 26‰ NaCl, were fed medicated feed containing 0.5% oxolinic acid to a feeding level of 0.5% of body weight divided into 4 times/day for 2 consecutive days followed by medicated feed every second day for 6 days (total of 6 days with medicated feed). The fish were starved between medicated feeding. Blood samples were taken at 3, 8, 24, 27, 32, 48, 51, 56 and 72, 96, 120, 144, 168, 192, 216 and 240 h after the commencement of the treatment.

An average serum concentration of 4 μg/mL was achieved between 2 and 3 days after the commencement of the treatment. During the treatment period, the lowest determined serum concentration of oxolinic acid (C_{min}) was 1.6 μg/mL (sample No. 149 taken at 216 h). The highest determined serum concentration of oxolinic acid (C_{max}) was 8.6 μg/mL.

The plasma profile after the oral multiple dosage regimen corresponded well with an estimated profile based solely upon the pharmacokinetics of the single dose study presented above, ie, C_{max} 4.1 μg/mL after 48 h and 3.9 μg/mL after 218 h. The dosage regimen used should be sufficient to maintain the serum levels of oxolinic acid between 3 - 5 μg/mL in fish sera.

Estimation of withdrawal time of oxolinic acid has also been reported by Hustvedt (1993c). A statistical approach to the setting of withdrawal time was used. It was suggested that the time intercept between detection limit of the analytical method and the upper 90% prediction limit of a linear regression on the logarithm of the serum concentration provided a realistic estimate of necessary withdrawal time as 35 days. Tissue samples were analyzed to test the suggested withdrawal time. Concentrations of oxolinic acid were followed in a group of fish post treatment. Blood samples from 10 fish were taken at 2, 6, 14, 21, 28, 35, 42 and 49 days after the end of the treatment. Muscle, liver and kidney samples from 8 of these fish were taken at 14, 21, 28, and 35 days post treatment.

At a mean temperature of 8.2°C, the level of oxolinic acid was predicted to fall below 1 ng/ml with a probability of 95% at 35 days post-treatment. The serum concentration of oxolinic acid was shown to fall below the detection limit of the analytical method within 28 days post treatment, corresponding values in muscle, liver and kidney were between 28 and 35 days. Tissue samples fell below the detection limit of the analytical method within the suggested 35 days. In contrast, Michel (1986) recommended a withdrawal time of only 6 days. Archimbault et al. (1988) also proposed the withdrawal time to be 6 days on the base of a tolerance level of 0.05 ppm following a dosage-level of 12 mg per kg per day for 7 consecutive days at 9-10°C. Jacobsen (1989) suggested a withdrawal time of 20 days after dosing at 10 mg per kg per day for 10 days in rainbow trout kept in freshwater at approximately 18°C *(vide infra)*.

It has been demonstrated (Ishida, 1990b) that the change in oxolinic acid levels in seawater-acclimatised coho salmon after oral administration was different from that in freshwater coho salmon. It was also found that the change in oxolinic acid levels in seawater coho salmon were similar to those in seawater fishes such as Japanese mackerel, red sea bream, yellowtail, and flounder. Oxolinic acid therefore appears to be retained at higher concentrations and for longer times in the freshwater fishes than in the seawater fish. Experiments with rainbow

trout *(vide infra)* supports this conclusion. It is suggested that the difference of the change in oxolinic acid levels after oral administration must be a function of the salinity of the fish's environment (Ishida, 1992).

Hustvelt et. al. have studied the therapeutic use of oxolinic acid in combatting the infection of Atlantic salmon infected with *Vibrio salmonicida,* the causative agent of cold-water vibriosis. Mean serum levels were maintained above 1.2 μg/mL for more than 10 days by oral treatments of 15 mg/kg body weight of oxolinic acid per day. A total dose of 75 mg oxolinic acid per kg fish was given at seawater temperatures between 2°C and 4°C (site 1), while a total dose of 90 mg per kg fish was given at seawater temperatures between 13°C and 15°C (site 2). Mortalities ceased between 3 and 4 days after initiation of treatment in both cases. Based on measured serum concentrations of oxolinic acid post-treatment, withdrawal periods of 38 days at 4-6°C and 31 days at 13-15°C were necessary to ensure (with a probability of 95%) that the drug levels would remain below 1 ng per mL serum.

In a comparison of the efficacy of oxolinic acid with that of nalidixic acid against *A. salmonicida* infections (furunculosis) Atlantic salmon were force fed with between 5 and 20 mg/kg of each drug (Parke-Davis, 1994). The first dose of medicated feed was followed by a second dose after 4 days. At all dose levels, oxolinic acid treated fish survived whereas there were mortalities in nalidixic acid treated fish, even at the highest dose. The oral administration of 10 mg oxolinic acid per kg fish to freshwater and seawater Atlantic salmon produced serum oxolinic acid levels which, in all cases, exceeded by many times the highest 48 hour MIC value of 0.03 mg/mL observed for *A. salmonicida* strains.

The serum level obtained in freshwater salmon following 7 daily consecutive feeds at 10 mg oxolinic acid per kg are in the same range as those obtained in seawater salmon as well as those of freshwater carp (Endo et. al., 1973). There is a marked variation in serum levels with individual fish but this can be expected due to a variable feeding response (Parke-Davis, 1994, p. 69).

Serum levels in freshwater salmon are higher in the samples taken after 10 consecutive daily feeds than those taken after the 7 consecutive daily feeds. This may be due to some accumulation of the drug although this was not evident in seawater, or it may be a reflection of a variable feeding response. The second possibility is supported by the wide range of values present. The test population of freshwater salmon parr in this experiment was much smaller than the normal population occupying a tank of the size used and this may well have affected the feeding response of the fish.

Oxolinic acid does not persist in edible salmon tissues beyond 7 days following a 10 day feeding period at 10 mg/kg, although a trace of oxolinic acid was recorded in the kidney tissue at 7 days post final treatment. Rapid elimination of oxolinic acid was also shown by Endo et. al. (1973) following oral administration to carp. From these results, a withholding period of 14 days is recommended between final treatment and culling of fish for human consumption.

An increase in the dose of oxolinic acid above 10 mg/kg leads to an increase in serum levels, but there is a levelling of serum concentration above a dose of 80-160 mg oxolinic acid per kg fish. The absence of mortality in fish receiving this dosage demonstrates that the serum levels attained are not toxic to the fish. The levelling off of the serum concentration also suggests that drug absorption may be restricted at oral doses in excess of 160 mg/kg.

From mammalian studies it was found that oxolinic acid is metabolised into a number of pharmacologically active metabolites. If it is assumed that in fish metabolism of oxolinic acid is similar, it would be impractical to chemically assay the sum of active metabolites in fish. Hence a microbiological assay method (Finlay and Stevenson, 1994) was chosen as a reliable method of indication of oxolinic acid level in the tissue, rather than other methods assaying pure oxolinic acid (Parke-Davis, 1994, p. 69).

Serum level data for oxolinic acid in Atlantic salmon found in this work is detailed in Tables 3 - 6.

Table 3. **Mean Serum Levels of Oxolinic Acid for Atlantic Salmon in Seawater Determined from Samples Taken at Various Time Intervals Following 6 Consecutive Daily Dietary Doses (Alderson and Finlay, 1994).**

OxolinicAcid Dose Rate (mg/kg)	Mean Serum Levels (μg/mL)			
	6h	24h	48h	72h
5	0.76	-	0.07	0
10	1.61	0.81	0.33	0.15
20	2.61	-	0.55	0.37

Table 4. **Mean Serum Levels of Oxolinic Acid for Atlantic Salmon in Seawater Determined from Samples Taken at Various Time Intervals Following 3 Daily Dietary Doses (Alderson and Finlay, 1994).**

Oxolinic acid Dose Rate (mg/kg)	Mean Serum Levels (μg/mL)		
	6h	24h	96h
40	2.8	3.6	n.d.
80	5.3	4.6	n.d.
160	7.2	5.2	n.d.
320	5.0	5.4	trace
640	6.1	4.8	n.d.

n.d.= not detected

Table 5. **Mean Serum and Tissue Levels of Oxolinic Acid for Seawater Atlantic Salmon after 10 Consecutive Daily Dietary Doses of 10 μg/kg (Finlay and Alderson, 1994).**

	Mean Oxolinic Acid Level (μg/mL)		
	6h	24h	72h
Serum	1.64	0.98	0.27
Liver	1.12	0.19	trace
Kidney	1.12	0.20	trace
Muscle	1.79	0.29	trace

Table 6. **Mean Serum and Tissue Levels of Oxolinic Acid for a Caged Population of Seawater Atlantic Salmon after 10 Consecutive Daily Dietary Doses of 10 μg/kg (n=5) (Rae et. al., 1994).**

	Mean Oxolinic Acid Level (μg/mL)		
Day after last dose	Day 1	Day 4	Day 7
Serum	1.39	trace	n.d.
Liver	0.40	trace	n.d.
Kidney	0.29	0.04	trace

n.d.= not detected

Brown Trout *(Salmo trutta)*

Rainbow Trout *(Oncorhynchus mykiss)*

In a study on rainbow trout kept in freshwater and in seawater at 8.5°C, elimination half-life, apparent volume of distribution and total clearance of oxolinic acid were estimated after a single intravascular injection. The drug was given as a rapid injection through a cannula into the dorsal aorta. Repeated blood samples were taken via the cannula and serum was analysed by reversed-phase high-performance liquid chromatography (Hustvedt and Salte, 1991).

The terminal elimination half-life for oxolinic acid in rainbow trout, estimated to be 52.6 h in freshwater ($t_{½\beta}$), was significantly lower than that in sea water (29.1 h). This considerable difference in elimination half-lives was mainly due to the increase in total clearance. Total clearance values were 1.2 and 2.0 $L \cdot kg^{-1}24h^{-1}$. The apparent volume of distribution was estimated to be 2.9 and 2.61 L/kg in freshwater and seawater, respectively. The large apparent volume of the central compartment in rainbow trout kept in freshwater indicated that oxolinic acid was initially rapidly distributed to tissues outside of the blood volume of the fish. Furthermore, the large apparent volume of distribution indicated that more than 90% of the drug in the fish was outside the serum.

The concentrations of oxolinic acid in tissues of seawater-acclimatised rainbow trout were compared with those of fresh water rainbow trout after a single oral administration (40 mg/kg). Up to 24 h after administration, little difference in the tissue concentrations was observed between the two groups of trout. However, tissue concentrations of the drugs in seawater trout decreased to undetectable levels by 72 h, whereas the concentrations in the freshwater trout peaked at 48 h and were detectable for at least 244 h. The kinetics of oxolinic acid uptake and excretion in the seawater trout were similar to those reported for seawater teleosts. Both groups of trout metabolized oxolinic acid by the same pathway, because the same oxolinic acid metabolites were observed in the bile of both groups of trout after a single oral administration. When oxolinic acid was injected (20 mg/kg) into the caudal vessels of each group of trout, serum levels of oxolinic acid decreased immediately after injection in both groups. Serum concentrations were 8.69 $\pm$ 2.14 μg/mL in the freshwater trout and 8.03 $\pm$ 2.27 μg/mL in seawater trout by 3 h after injection, respectively. Subsequently, the serum levels of oxolinic acid in the seawater trout decreased to barely detectable levels by 72 h. In contrast, oxolinic acid levels in freshwater trout were higher and persisted for longer, up to 244 h. These results confirm that oxolinic acid is excreted more rapidly in seawater trout than in freshwater trout.

Endo et al. (1987) had previously determined that there was a difference in the elimination of oxolinic acid between different fish species by comparing their data for yellowtail with data reported for rainbow trout and ayu by Kasuga et. al. (1984)

The pharmacokinetics and bioavailability of oxolinic acid were studied in rainbow trout at a water temperature of 16°C after intravascular and oral dosing (Bjorklund and Bylund, 1991). 10 Fish weighing 514 $\pm$ 14 g were given an intervascular dose of 10 mg/kg oxolinic acid and 5 fish weighing 501 $\pm$ 20 g were dosed orally at 75 mg/kg in this work.

The pharmacokinetics were best described by a two-compartment open model giving distribution half-lives of 0.31 h and 1.53 h, and an elimination half-life of 69.7 h for oxolinic acid with a volume of distribution of 1.94 L/kg. The elimination half-life, total clearance time and apparent volume of distribution was generally in line with the work of Hustvedt and Salte (1991). The apparent oral bioavailability for oxolinic acid was 13.6% and the plasma protein binding was 27%. The drug was well tolerated with an acute oral toxicity (LD_{50}) exceeding 4000 mg/kg.

The temperature-related absorption and excretion of oxolinic acid in rainbow trout has been studied by Bjorklund et. al. (1992). Absorption and elimination in serum, bile and tissues were studied at 5, 10 and 16°C in fresh water fish weighing 266 $\pm$ 48 g after a single oral dose of 75 mg/kg oxolinic acid. The maximum levels of oxolinic acid were obtained in serum within 1, 4 and 6 days at 16, 10 and 5°C, respectively. The highest drug concentrations were measured in bile followed by liver, kidney, muscle tissue and serum. The higher levels of oxolinic acid in tissues compared to serum indicate a good distribution of the drug. The elimination half-life in serum was 24 h at 16°C, 4.0 days at 10°C and 6.1 days at 5°C. With a detection limit of 0.01 μg/g for the oxolinic acid in the HPLC assay, the predicted withdrawal time with 95% confidence for muscle tissue was 28 days at 16°C, 60 days at 10°C and 140 days at 5°C. Results obtained under laboratory conditions were similar to those from field trials. These results are summarised in Table 7.

Table 7. **Elimination Rate Constants (β), Half-lives ($t_{½}$) and Predicted Withdrawal Times (t_{pred}) for Oxolinic Acid in Tissues of Rainbow Trout at Different Temperatures.**

Tissue	Temp (°C)	$\beta \pm$ SD/day	$t_{½} \pm$ SD (days)	t_{pred} (days)*
Serum	5	0.114 ± 0.010	6.1 ± 0.8	125
	10	0.171 ± 0.028	4.0 ± 1.0	54
	16	0.711 ± 0.033	1.0 ± 0.06	15
Muscle	5	0.103 ± 0.016	6.7 ± 1.5	140
	10	0.151 ± 0.007	4.6 ± 0.3	60
	16	0.354 ± 0.037	2.0 ± 0.3	28
Liver	5	0.091 ± 0.013	7.6 ± 1.6	270
	10	0.102 ± 0.010	6.8 ± 1.0	165
	16	0.186 ± 0.015	3.7 ± 0.4	60
Kidney**	16	0.192 ± 0.010	3.6 ± 0.3	44

Fish were given a single oral dose of 75 mg/kg oxolinic acid/kg body weight. Each value is a mean of five fish.

* The predicted withdrawal times (with 95% confidence) are based on a detection limit of 0.01 μg/g for the HPLC assay.

** Levels of oxolinic acid in kidney were not measured at 5 and 10°C.

Oxolinic acid , oxytetracyline and trimethoprim were investigated in whole gutted rainbow trout together with their skin, muscle and blood, at 6, 12 and 18°C. Concentration of drugs were measured by HPLC. It is recommended that wilhdrawal times for rainbow trout be based on whole gutted rainbow trout and not, as in earlier investigations, from residual concentrations in muscle. Oxolinic acid in rainbow trout feed was found to be stable during the pelleting process used and up to 81 days after pelleting. The absorption of the drug in rainbow trout shows different temperature dependencies. A quantitative comparison data from this work with individual results from earlier investigations suggested withdrawal times for freshwater rainbow trout of about 20 days at approximately 18°C.

Archimbault et. al. (1988) studied the serum and tissue concentrations of oxolinic acid in rainbow trout (approximate weight 200 g) fed orally at a rate corresponding to 12 mg/kg/day for 7 consecutive days. This dosing allowed a maintenance of tissue and serum levels of oxolinic acid in excess of appropriate MICs for two fish pathogens. At the end of the treatment period, tissue concentrations of 1.97 μg/g were found which had declined to 0.05 μg/g at 6 days and 0.03 μg/g at 10 days after withdrawal of the medicated feed in fish maintained at 9-10°C.

The prophylactic efficacy of oxolinic acid against *A. salmonicida*-infection of rainbow trout has been investigated (Parke-Davis, 1994). Two groups of fish, an 0+ group (100 x 3.5-5 g) and a 1+ group (30 x 600-650 g), maintained in aerated freshwater at 18°C were administered oxolinic acid at doses of 10 mg/kg for 10 days. All of the fish survived at all dosing levels whereas 56% of 0+ and 50% of 1+ control fish had died 9 days after challenge.

The efficacy of oxolinic acid in the protection of brown trout challenged with *A. salmonicida* infections has been established (Parke-Davis, 1994). Fish, maintained in aerated freshwater at 18°C, were administered oxolinic acid incorporated into the food for 10 days, commencing at 4 days post-challenge with *A. salmonicida* . At doses of 75 and 50 mg/kg, all of a group of 50 fish survived and, at 5 and 25 mg/kg, only one of a group of 50 fish died. By contrast all fish in the untreated control group died.

In a parallel study (Parke-Davis, 1994), the prophylactic efficacy of oxolinic acid against *A. salmonicida* infection of brown trout was investigated. Groups of 50 fish, maintained in aerated freshwater at 18°C, were administered oxolinic acid at doses of 5, 25, 50 and 75 mg/kg. All of the fish survived at all dosing levels whereas all of the 50 control fish had died 10 days after challenge.

Eels

The *in vitro* antibacterial activity of oxolinic acid on diseased eels has been studied (Liu, 1991). Pharmacokinetics of oxolinic acid administered to eels was studied together with its efficacy against artificial infections of *Aeromonas hydrophila* and *E. tarda.*

Results obtained from an *in vitro* antibacterial study showed that minimal inhibitory concentrations of oxolinic acid against *Aeromonas hydrophila, E. tarda* and *Pseudomonas anguilliseptica* were 0.10-0.78, 0.10-0.39, and 0.10-0.20 μg/mL, respectively; whereas those against *Flexbacter columnaris* were 6.25-25.1 μg/mL. In an efficacy trial, eels were artificially infected with *A. hydrophila* and *E. tarda,* respectively, and oxolinic acid was administered on each 3rd consecutive day with a single dose of 8, 12 and 16 mg/kg body weight per day, respectively. The results showed that a significant difference ($p<0.05$) of the cumulative mortalities was obtained between the control group and the groups treated with 12 mg/kg b.w. or those with 16 mg/kg bw ($p<0.05$). No pathogens were isolated from the survivors for each medicated group. However, tested bacteria could be isolated from the survivors of the control eels.

In a pharmacokinetic study, 3 time course patterns for various concentrations of oxolinic acid in serum and tissues were found. In the first pattern, the highest concentration in serum, liver and muscle occurred 3 hours post-administration. The concentration of drug in serum and tissue then declined and the second concentration peak was detected at 12 hours after oral administration. Subsequently the drug concentration decreased with time. In the second pattern, the maximum concentration in gill was obtained at 6 hours post-administration and then declined rapidly with time. In the third pattern, highest drug concentration in kidney was found at 6 hours post-administration followed by a slow decrease. The second concentration peak in kidney was detected at 24 hours after oral administration which decreased gradually with time.

Twelve days after administration of oxolinic acid at a dosage level of 12 mg/kg bw for 3 consecutive days, no drug residue was detected, either in eel serum or tissue.

Chickens

Archimbault and Ambroggi (1987) investigated the use of oxolinic acid in poultry. A serum bioavailability study was performed after oral administration in hens of a single dose of 10 mg/kg and after repeated doses of 15 mg/kg/day for 5 consecutive days. Lung concentration and residue levels in eggs were also evaluated after administration of repeated doses using an analytical method with a detection limit of 10 ng/g or mL. Overall studies confirmed the therapeutic potency in poultry of a dose of 15 mg/day and gave an estimation of the oxolinic acid residues in tissues and eggs. If a maximum residue limit of 50 μg/kg is adopted, the elimination of oxolinic acid requires a withdrawal period of 6 days for tissues and 8 days for eggs.

Anadon et. al. (1990) studied the pharmacokinetics and residue depletion of a series of quinolone compounds and of olaquindox in poultry. Oxolinic acid (15 mg/kg) fed orally to 8 x 40 day old broiler chickens (Hubbard x Hubbard, weighing 2.5 kg) gave the following mean pharmacokinetic parameters: T_{max} (h) 2.7 ± 0.14, C_{max} (μg/mL) 11.93 ± 0.29, $t_{½}\beta$ (h) 33.54 ± 9.88, and AUC (mg·h/L) 478.10 ± 33.10.

Mean tissue concentrations of oxolinic acid following oral administration of 200 mg/kg to 6 x 40 day old broiler chickens (Hubbard x Hubbard, weighing 2.5 kg) are shown in Table 8.

Table 8. Mean Tissue Concentrations of Oxolinic Acid Following Oral Administration of 200 mg/kg to 6 x 40 Day Old Broiler Chickens (Hubbard x Hubbard, Weighing 2.5 kg)

Tissue	Oxolinic Acid Concentration (μg/g)			
	Day 1	*Day 3*	*Day 6*	*Day 8*
Muscle	1.46 ± 0.16	0.57 ± 0.15	0.02 ± 0.01	n.d.
Liver	2.16 ± 0.13	0.49 ± 0.06	0.05 ± 0.01	n.d.
Kidney	2.38 ± 0.35	0.91 ± 0.45	0.16 ± 0.05	0.05 ± 0.01

n.d. = not detected. Quantification by HPLC (Horie et. al., 1987)

Based on these results, the authors suggest 8 days as an appropriate withholding period for poultry after cessation of medicated feed.

METABOLISM

Introduction

Oxolinic acid has been a widely used prophylactic and chemotherapeutic agent in aquaculture over a decade and the pharmacokinetics of the substance have been studied in fish and animals as well as man. The few metabolic studies of oxolinic acid which have been carried out were relatively early works and the structure of many of the metabolic products were not elucidated.

Although the pharmacokinetic behaviour of oxolinic acid has been well studied in many fish species, the metabolism of the drug in these species has not been studied in any depth and the fate of the drug is largely a matter of conjecture. In the absence of any definitive metabolism study in fish, the known metabolism in other species are discussed below. This does not necessarily indicate that the metabolism in fish is similar.

Metabolism in Man

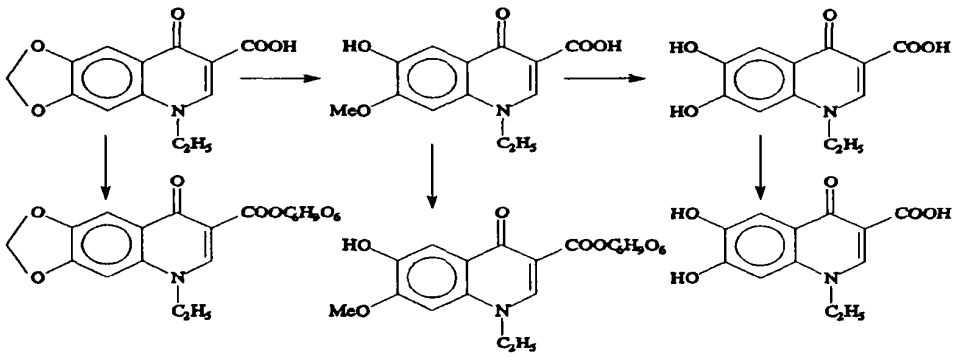
Human metabolism of oxolinic acid is considered here because of a general dearth of information about its metabolism in those animal and fish species where it is most widely used. In man (4 subjects treated with a single 1 g oral dose of drug), oxolinic acid is converted into at least 8 metabolites which are excreted principally as glucuronides (Di Carlo et. al., 1968 a,b). The peak blood radioactivity was observed 4 hours post-dosing when 1.2% of the radioactivity was in the systemic circulation. The elimination of radioactivity into the urine and faeces averaged 42.7% for the first 24 hours and 66.7% over the whole study period of 48 hours. Both urine and faeces demonstrated antimicrobial activity although free oxolinic acid was not present. The radioactivity in the zero to 6 hour collections was found to consist of 43.4% oxolinic glucuronide, 1.4% labile complex of oxolinic acid, 37.5% glucuronides derived from modified oxolinic acid and 17.7% non-glucuronide metabolites.

The glucuronides of both parent drug and some metabolites are unstable in aqueous solution, Thus in the case of oxolinic acid glucuronide, the active parent drug is readily released. It would appear, however, that only 10% of the administered dose reaches the urinary tract in an unmetabolised state (Graber et. al., 1974).

In the 24 hours post-dosing, 45% of radiolabelled oxolinic acid is excreted by man via urine (35%) rather than through the faeces (10%), but none is excreted in as the parent drug (Crew et. al., 1971).

The major metabolites of oxolinic acid in man are summarised in Figure 1 (Graber et. al., 1974). Comparative studies on the excretion of radiolabelled oxolinic acid by rabbit, rat, dog and man revealed that all four species metabolised oxolinic acid in much the same way. However, quantitatively, none of the animal species disposed of oxolinic acid as did man. Of particular interest was the cleavage of the methylenedioxy-ring of oxolinic acid, the first time the cleavage of such a ring had been established in man (Crew et. al., 1971).

Figure 1. Human Metabolism of Oxolinic Acid



Metabolism in Rabbits, Rats and Dogs

In a comparative study of oxolinic acid metabolism, the excretion and metabolic pathways of this substance was studied in rabbits, rats and dogs (Crew et. al., 1971) and the results compared to an ealier study on oxolinic acid metabolism in man (Di Carlo et. al., 1968 a,b). After a single dose of radiolabelled oxolinic acid, at 10 mg/kg the excretion of radioactivity and individual component identification was carried out as throughly as possible. The age of this study precludes the accuracy of identification which could be acheived today. The results of this study are summarised in Table 9.

Table 9. Composition of Urinary Radioactivity after Oral Administration of ^{14}C-Oxolinic Acid (10 mg/kg)*

			Percentage of dose of radioactivity							
			Unconjugated			Glucuronides				
Species	Time Interval (h)	No. of Animals	OA	CM	Total	OA	CM	Total	Not known	Total ^{14}C
Rat	0-6	5	1.5	0.3	2.0	2.2	0.9	4.0	4.5	10.5
Rat	0-6	4	3.4	0.3	3.9	0.4	0.8	1.7	8.1	13.7
Dog	0-6	1	0.1	0.04	0.3	1.4	0.1	3.2	3.9	7.4
Man*	0-6	4	0.3	<0.01	0.4	7.8	3.4	12.9	4.6	17.9
Rat	0-24	5	2.3	0.7	3.3	4.5	2.1	8.9	11.4	23.6
Rat	0-24	4	5.6	0.9	7.4	1.0	2.0	4.3	19.1	31.4
Dog	0-24	3	0.3	0.2	1.0	1.8	1.0	7.0	10.5	15.5
Rabbit	0-24	4	3.8	1.0	4.9	4.9	5.0	21.9	22.1	48.9

* Dose in man was 1.0 g/subject; OA = oxolinic acid; CM = catechol metabolite (1-ethyl-1,4-dihydro-7-hydoxy-6-methoxy-4-quinolone-3-carboxylic acid

Metabolism in Fish

Metabolites in bile of rainbow trout

In a study of the metabolic pathway of oxolinic acid in fish (Ishida,1992), the metabolites of oxolinic acid in the bile of seawater and freshwater trout were compared. Oxolinic acid (OA), two glucuronides of its decomposition products (7-OH-OA-G, 6-OH-OA-G) and oxolinic acid glucuronide (OA-G) were detected in the bile of both groups of trout. The relative concentrations of oxolinic acid and three glucuronides are shown for the two groups of fish in Table 10. Unchanged oxolinic acid was the dominant component 6 h after treatment in both groups. However, 24 h after treatment, oxolinic acid glucuronide was the dominant component, accounting for 66.2 $\pm$ 3.5% for the freshwater trout and 65.7 $\pm$ 7.9% for the seawater trout. The relative amounts of the four compounds in the bile of seawater trout after 24 h were similar to those of freshwater trout (Table 10).

Table 10. Relative Proportions of Oxolinic Acid and its Metabolites in the Bile of Freshwater and Seawater Rainbow Trout after a Single Oral Administration (40 mg/kg)[a]

Fish	Hours	OA (%)	6-OH-OA-G (%)	7-OH-OA-G (%)	OA-G (%)
Freshwater rainbow trout	6	62.0 ± 30.1	n.d.	n.d.	38.0 ± 30.8
	12	58.1 ± 36.3	n.d.	n.d.	41.9 ± 36.3
	24	28.8 ± 1.2	1.8 ± 1.3	3.4 ± 3.1	66.2 ± 3.5
Seawater rainbow trout	6	50.2 ± 12.1	n.d.	n.d.	49.8 ± 12.1
	12	28.0 ± 8.6	7.0 ± 8.3	9.4 ± 8.1	55.6 ± 8.2
	24	22.4 ± 6.5	4.6 ± 5.5	7.3 ± 8.6	65.7 ± 7.9

[a] Mean ± SEM (n=4); OA = oxolinic acid; 7-OH-OA-G = 7-hydroxy derivative glucuronide; 6-OH-OA-G = 6-hydroxy derivative glucuronide; OA-G = oxolinic acid glucuronide; n.d.= not detected.

TISSUE RESIDUE DEPLETION STUDIES

General

Tissue residue depletion studies of oxolinic acid based on the use of tritium labelled compounds have seldom been carried out. All investigations published to date use the rate of disappearance of the parent drug to monitor the rate of tissue depletion. Therefore tissue depletion studies and pharmacokinetics of the parent drug are often dealt with in the same report and recommended withdrawal periods are specifically based on the rate of disappearance of parent drug (active metabolites are seldom discussed). Where depletions studies and pharmacokinetics have been discussed in the same report, they are discussed in the Section on pharmacokinetics (*vide supra*).

METHODS OF ANALYSIS FOR RESIDUES IN TISSUES

General

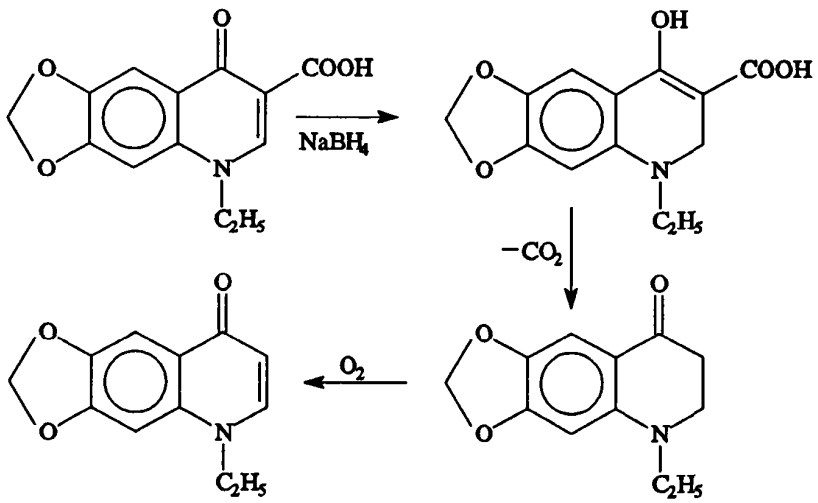
Because of the wide spread use of oxolinic acid, particularly in the aquaculture industry, there are a plethora of publications detailing analytical procedures to quantitate residues of oxolinic acid and related quinolone antimicrobials. All published methods in food matrices measure the concentration of free oxolinic acid in that matrix.

Gas Chromatography - Mass Spectrometry

There appears to be only one direct method for oxolinic acid analysis based on gas chromatography-mass spectrometric detection (Takaksuki, 1992). Oxolinic acid and other quinolone acids are extracted with ethyl acetate from fish homogenate buffered at pH 6, back-extracted in bicarbonate, neutralised and again taken up in ethyl acetate. The solvent is evaporated and the residue treated with methanolic sodium borohydride to give decarboxy-derivatives by a process illustrated in Figure 2. The decarboxy-derivatives were then analysed by gas chromatography - mass spectrometry in the SIM mode with a detection limit of 3 ng/g. Recoveries of 80% were obtained for oxolinic acid from fish muscle fortified at the 0.01 ppm level.

This method has been adapted (Pfennig et. al., 1993) and used as a confirmatory technique applied to samples where the presence of oxolinic acid was first indicated by HPLC (*vide infra*). It is the only report of the direct confirmation, (based on SIM of 4 ions) of the presence of oxolinic acid residues detected by HPLC in the literature at present. However, an off-line method (Maddock et al, 1983) to establish peak identity in HPLC analysis has also been reported (*vide infra*).

Figure 2. Formation of Decarboxy-oxolinic Acid for GC-MS Determination



Decarboxyoxolinic acid

Biological Tests

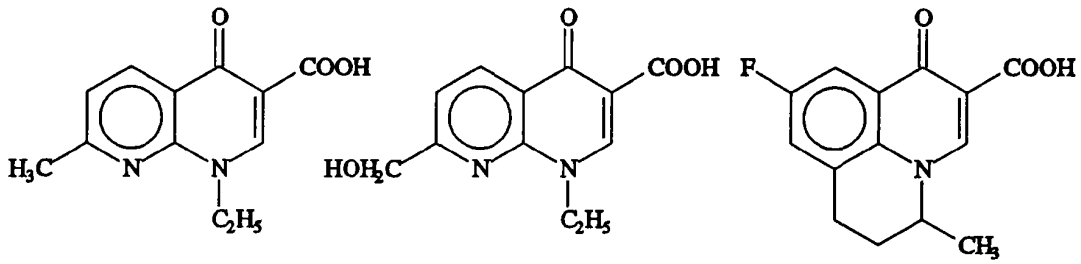
Biological tests based on microbial activity have been developed and applied in a number of studies but suffer from a general lack of sensitivity. Thus a lower limit of detection of only 0.25 μg/mL is achievable with fish plasma or prawn haemolymph (Limpoka el. al., 1994).

High Performance Liquid Chromatography (HPLC)

A number of high performance liquid chromatographic methods are fully validated for a range of fish and animal tissues. Many of these methods appear to offer the potential sensitivity and ruggedness to be adaptable to a wide range of biological matrices. These potentially useful methodologies are therefore included in the review of analytical procedures for oxolinic acid.

Several methods for the detection and quantitation of oxolinic acid by HPLC with UV detection have been documented.

Figure 3. Substances Used as Internal Standards in Oxolinic Acid Analysis



Nalidixic acid Hydroxynalidixic acid Flumequine

An HPLC procedure based on a reverse phase chromatographic separation with UV detection has been successfully developed to monitor oxolinic acid in plasma and tissues of trout and salmon. Hydroxynalidixic acid was utilised as the internal standard for the method. Sample clean-up involved extraction of oxolinic acid into chloroform from tissue homogenates buffered to pH 5.0, evaporation of the solvent and reconstitution of the residue in a pH 6.0 buffer followed by further purification by column chromatography prior to quantitative

determination by reverse-phase HPLC using a buffered methanol with added ion pairing agent (cetyl trimethylammonium bromide) with UV detection at 315 nm. Oxolinic acid could be determined in various tissues, the limit of quantification of the analytical method ranging from 21 ng/g in liver to 68 ng/g in skin, which was the most difficult matrix to analyse (Maddock et al, 1983). The average recovery of hydroxynalidixic acid, the internal standard, was 60-70%. Off-line confirmation of HPLC peak identity could be obtained by collection evaporation of the appropriate HPLC fraction followed by mass spectrometry (Maddock et al, 1983).

An earlier procedure using reverse-phase HPLC separation of oxolinic acid from fish plasma (Rees and Lewis, 1982) proved unsuitable for determination of oxolinic acid residues in tissue samples (Maddock et. al., 1983).

Another reverse-phase HPLC procedure for the isolation and determination of oxolinic acid from fish serum employed either liquid or solid phase extraction (Hustvedt et. al., 1989). After addition of an internal standard (nalidixic acid), the serum was acidified with hydrochloric acid and extracted with a 1:1 mixture of chloroform and ethyl acetate or applied directly to a solid phase cartridge. The cartridge was washed with sodium hydroxide and the oxolinic acid and internal standard eluted with citrate buffer and methanol. The elution solvent was buffered aqueous methanol containing an ion pairing agent (tetrabutylammonium phosphate) with UV detection at 258 nm. The detection limit for oxolinic acid by this method was 1 ng/mL while the Coefficient of Variation (CV) at 1 μg/mL was 17.5% for the solid phase extraction but only 3 % for liquid extraction. The CV for solid phase extraction was lowered to less than 3% by repeated use of a single extraction cartridge.

The simultaneous extraction and determination of oxolinic acid and flumequine in fish tissues by high performance liquid chromatography has also been reported (Rogstad et. al., 1989). This study compared the extraction efficiency of oxolinic acid and flumequine by organic solvents and solid phase extraction, respectively. Flumequine was used as an internal standard for the determination of oxolinic acid, whilst oxolinic acid served as the internal standard for the determination of flumequine. HPLC was conducted on a resin column under acidic conditions using a mobile phase of acetonitrile, tetrahydrofuran and aqueous phosphoric acid with fluorescence detection. The limit of quantification in muscle and liver was 0.5 ng/g for oxolinic acid and 2 ng/g for flumequine. The study showed that the efficiency of extraction was lower with a solid phase extraction (SPE) cartridge than with solvent, while the CVs for the analyses were similar for either extraction method. It was concluded that use of SPE did not appear to be more cost effective than solvent extraction. Comparative data for the efficiency of the two extraction methods for fish muscle are shown in Table 11.

A rapid and highly sensitive method for the quantitative determination of oxolinic acid and flumequine in salmon plasma by high-performance liquid chromatography with fluorescence detection has been developed (Samuelsen, 1990). As in the previous method, flumequine was used as an internal standard for the determination of oxolinic acid whilst oxolinic acid served as the internal standard for the determination of flumequine. Protein in the fish plasma was precipitated with zinc sulfate and acetonitrile and, after centrifugation, the supernatant was analysed directly on a C_8 reverse phase column using a complex mixture of solvents buffered to pH 3.2 with oxalic acid. Recoveries were in excess of 90% for both analytes at 50 ng/mL whilst limits of detection were 3 and 5 ng/mL for oxolinic acid and flumequine, respectively.

Two separate methods for the determination of oxolinic acid and flumequine in salmon plasma have been described (Rasmussen et. al., 1989a,b). In the first method, the sample is applied to a disposable C_2 solid phase extraction column. Nalidixic acid was added as the internal standard and the analytes eluted from the C_2 column using a mixture of acetonitrile, methanol and 1M phosphoric acid. In the second method the plasma was injected directly onto a polystyrene-divinylbenzene precolumn for sample cleanup. The precolumn was eluted with dilute phosphoric acid until interfering substances were removed followed by elution of the analytes onto the analytical column with the mobile phase. HPLC conditions were common to either cleanup procedure, utilising a resin-based column under acidic conditions with a mobile phase of acetonitrile, tetrahydrofuran and aqueous phosphoric acid with fluorescence detection as previously reported (Rogstad et. al., 1989). The limit of determination for the column switching procedure was 5 and 10 ng/mL for oxolinic acid and flumequine, respectively. The recovery and reproducibility of analysis of oxolinic acid by both isolation methods is shown in Table 12.

Table 11. Comparative Data for the Isolation of Oxolinic Acid and Flumequine from Fish Muscle by Solvent Extraction and Solid Phase Extraction (Rogstad et. al., 1989)

Solvent Extraction

Tissue	Number of Samples	Spike Level (μg/g)	Recovery (%)			
			Oxolinic Acid		Flumequine	
			Mean	SD	Mean	SD
Muscle)	10	0.1	97	3.7		
(5 g)	6	0.01	97	7.2		
	8	0.5			92	3.8
	6	0.1			89	3.9

Solid Phase Extraction

Tissue	Number of Samples	Spike Level (μg/g)	Recovery (%)			
			Oxolinic Acid		Flumequine	
			Mean	SD	Mean	SD
Muscle	8	0.1	78.4	2.2		
(5 g)	8	0.5			77.1	3.8

SD = Standard Deviation

Table 12. The Recovery and Reproducibility of Analysis of Oxolinic Acid in Fish Plasma by Solid Phase Extraction and Column Switching Cleanup Techniques (Rasmussen et. al., 1989a,b)

Conc. of oxolinic acid (μg/mL)	Solid Phase Extraction Cleanup Technique		Column Switching Cleanup Technique	
	Mean Recovery (%) (n=6)	CV (%)	Mean Recovery (%) (n=6)	CV (%)
0.05			97.5	2.7
0.5	103.6	4.2	97.2	1.0
1.0	100.1	2.5	98.1	1.1
2.5	100.4	2.8	98.1	1.9
5.0	98.7	1.2	95.7	1.0

The poor adsorption of oxolinic acid by fish has raised the possibility that it could become an environmental contaminant. A reverse phase HPLC method for the determination of oxolinic acid with UV detection has been reported (Pouliquen et. al.,1994) with the object of studying the occurence, persistence and metabolism of oxolinic acid in seawater, marine sediment and Japanese oyster. Linearity and precision were satisfactory over a concentration range of 0.05-2.5 μg/mL or μg/g with limits of detection and determination of 0.01 and 0.04 μg/mL or μg/g respectively. Recoveries of oxolinic acid from spiked samples were 102.1% from seawater, 68.1% from marine sediment and 88.5% from oyster. The analyte was stable in all matrices at -20°C for 60 days. No analyses of actual environmental samples were reported in this work.

Pfennig et. al. (1993) report the determination of 4 quinolone antimicrobials including oxolinic acid for salmon tissue using acetone extraction, sample cleanup using solvent partition and HPLC determination using a PLRP-S polymer column and fluorescence detection. A similar multi-residue method for several antibiotic classes including oxolinic acid has been published by Nose et. al. (1987) using acetone extraction and alumina cleanup followed by reverse-phase HPLC with UV detection.

APPRAISAL

It has been demonstrated (Ishida, 1990b) that the change in oxolinic acid levels in seawater-acclimatised coho salmon after oral administration was different from that in freshwater coho salmon. It was also found that the change in oxolinic acid levels in seawater coho salmon were similar to those in seawater fishes such as Japanese mackerel, red sea bream, yellowtail, and flounder. Oxolinic acid therefore appears to be retained at higher concentrations and for longer times in the freshwater fishes than in the seawater fish. Experiments with rainbow trout *(vide supra)* supports this conclusion. It is suggested that the difference of the change in oxolinic acid levels after oral administration must be a function of the salinity of the fish's environment (Ishida, 1992). It is also clear that retention of oxolinic acid by some fish species is dependent on water temperature.

Therefore, the utilisation of oxolinic acid in aquaculture must be determined predominantly by its mammalian toxicity rather than by any inherent *in vivo* fish data.

Like many xenobiotic antimicrobial agents, oxolinic acid appears to be benign with respect to human health. The use of this drug in fish husbandry is therefore supported with an appropriate wihdrawal time. This wihdrawal time must be linked both to the water salinity and the water temperature utilised in fish aquaculture practices.

Maximum Residue Limits

The Committee was not able to set MRLs because no ADI was established. No additional residue data were requested.

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