SARAFLOXACIN

First draft prepared by
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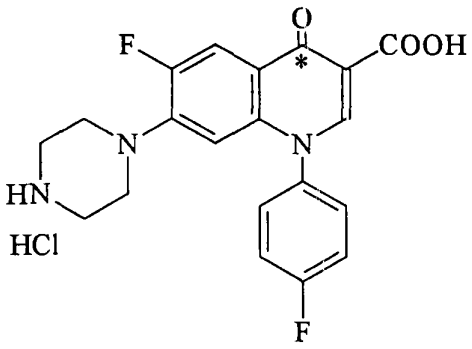
IDENTITY

Chemical name: Sarafloxacin hydrochloride; 6-fluoro-1-(4-fluorophenyl)-7-piperazinyl-1,4-dihydro-4-oxo-3-quinolinecarboxylic acid hydrochloride.

Synonyms: Floxasol

Structure:



* ^{14}C-4 for radiometric studies >98% pure

Molecular formula: $C_{20}H_{18}ClF_2N_3O_3$

Molecular weight: 421.8 (hydrochloride) 385.4 (free base)

OTHER INFORMATION ON IDENTITY AND PROPERTIES

Pure active ingredient: sarafloxacin hydrochloride

Appearance: white to pale yellow powder

Melting point: >300°C

Solubility (g/L): water, 0.31; methanol, 3.2; ethanol, 0.23; 1*N* NaOH, 165; DMSO, 8.8, practically insoluble in 1M HCl, chloroform, hexane, toluene.

Ultraviolet maxima: 261 nm and 317 nm

Factors affecting stability: the hydrochloride is stable in neutral, acidic and basic solutions; sarafloxacin is stable for 30 days at 110°C; in solution, sarafloxacin can be degraded by light and oxidising agents.

RESIDUES IN FOOD AND THEIR EVALUATION

CONDITIONS OF USE

General

Administered in drinking water for turkeys and broilers as an antibiotic compound.

Dosage

For chickens: 20 mg/L in water; equivalent to 4 mg/kg BW for chickens from 3 weeks of age.

For turkeys: 30 mg/L in water; equivalent to 4 mg/kg BW for turkeys from 7 weeks of age.

PHARMACOKINETICS AND METABOLISM

Pharmacokinetics

Toxicological Test Species

Compliance with Good Laboratory Practice principles was not required for these pharmacokinetic studies. The quality and design of this study was consistent with current scientific standards.

Mice

Three groups of twelve female mice per group were dosed with sarafloxacin ^{14}C-base as follows: Animals in the first two groups were given a single oral dose of 10 mg sarafloxacin/kg BW. One group received the drug by IV administration and the other via gavage. Animals in the third group were given a dose of 100 mg sarafloxacin/kg BW by gavage. Urine and feces were collected from the mice daily for three days.

Estimates of absorption of the parent drug were derived from data on 0 - 24 h urinary excretion. For the 10 mg/kg BW dose, 48% (range 27-73%) of the parent drug was absorbed. For the 100 mg/kg BW dose, 34% (range 29-38%) of the parent drug was absorbed.

Within 3 days after administration of a single IV dose of 10 mg/kg BW of sarafloxacin to mice, 49% of the ^{14}C-dose was excreted in the urine and approximately 44% was eliminated in the feces. Following oral administration of the same dose, urinary and fecal excretion accounted for approximately 25% and 80%, respectively. Mice given the 100 mg/kg BW oral dose eliminated 18% of that dose in the urine and 74% in the feces. Almost all of the radioactivity was excreted during the first 24 hours after either oral or IV administration (Volume 4a).

Rats

Six groups of Sprague Dawley rats (18/sex/group) were dosed with sarafloxacin as follows: One group of animals received a single IV dose of 20 mg/kg BW of sarafloxacin. Four groups of animals received a single oral dose of 20, 75, 275 or 1000 mg/kg BW of sarafloxacin. Animals in the sixth group received an oral dose of 1000 mg/kg BW of sarafloxacin daily for 14 consecutive days. Blood samples (4 rats/group) were collected just prior to dosing and at 0.5, 1, 2, 4, 6, 8, 12 and 24 hours post dosing on day 1 for the groups receiving the single dose and on days 1 and 14 for the 14 day dosing group. Plasma and urine samples were assayed for sarafloxacin base by a HPLC method. The pharmacokinetic parameters determined from this study are presented in Table 1. A comparison of the 0 to infinity AUCs following a single IV or oral 20 mg/kg BW dose of sarafloxacin indicated that the bioavailability was approximately 12% at this dose level. A plot of the AUC vs dose is linear up to 275 mg/kg BW (Volume 4b).

Table 1. Pharmacokinetic parameters of sarafloxacin in rats.

Dose (route) (mg/kg BW)	V_d (l/kg)	$T_{½}$ (elim) (h)	T_{max} (h)	C_{max} (mg/L)	k_a (h^{-1})	k_e (h^{-1})	ABC (mL/min/kg)
20 (IV)	5.3	2.0	-	-	-	0.3	30
20 (oral)	60	3.0	1.0	0.3	3.0	0.3	270
75 (oral)	70	2.0	2.0	0.6	1.0	0.4	470
275 (oral)	250	7.0	2.0	0.9	2.0	0.1	420
1000 (oral)	400	6.0	1.0	2.0	2.0	0.1	820
1000 (oral)*	110	6.0	2.0	8.0	1.0	0.1	200

ABC is apparent body clearance. * once daily for 14 days.

Rabbits

The absorption, metabolism and excretion of ^{14}C-labelled sarafloxacin was studied in 3 month old female New Zealand white rabbits. Two groups of 3 animals per group were dosed orally, by gavage, with 10 mg/kg BW of ^{14}C-sarafloxacin base. A third group of 3 animals received this same dose by IV administration. Blood samples were collected at 1, 3, 6, 12 and 24 hours after oral administration from animals in one of the groups dosed orally. Urine and feces were collected daily for five days from animals in the other oral dose group and the IV dosed group. Within 5 days after oral administration about 11% of the dose was eliminated in the urine and approximately 79% was eliminated in the feces. Urinary excretion following IV administration was used to determine that approximately 16% of the oral dose was systemically absorbed (volume 4c).

Dogs

Three groups of 14 dogs/group (species, age, sex not stated) were administered daily oral doses of 5, 25 or 125 mg/kg BW sarafloxacin base by capsule. After one month 6 dogs/group were killed and plasma and cerebrospinal fluid were collected for HPLC analysis. The remaining dogs continued to be treated daily for a total of 90 days. The pharmacokinetic parameters determined from this study are presented in Table 2.

Table 2. Pharmacokinetics of sarafloxacin base after oral administration to dogs.

Dose (mg/kg)	Mean half-life (h)[1]			AUC (mg·h/L)		
	2 doses	24 doses	79 doses	2 doses	24 doses	79 doses
5	5	6	6	9	9	10
25	5	5	6	30	31	30
125	5	6	6	104	108	106

[1] Samples were taken 1, 3, 6, and 24 hours after dosing.

For the low and mid-dose groups, peak serum levels of sarafloxacin were found most often in samples taken 3 hours after dosing. In the high-dose group maximum serum levels were found in the majority of animals 6 hours after dosing. Therefore the true half lives may be overestimated and the AUC values may be underestimated for some of the high-dose animals. Dose normalization of the AUC provides a gauge of dose-proportionality of systemic exposure. The trend of decreasing values of approximately 2, 1 and 1 μg·h/mL per mg/kg for the 5, 25 and 155 mg/kg BW dose groups, respectively, suggests that absorption efficiency is reduced with increasing dose size.

In summary, these data suggest that the dispositional kinetics of sarafloxacin in the dog are independent of dosage size and treatment duration while absorption of sarafloxacin becomes less efficient with increasing dose size (Volume 4d).

Tissue distribution of ^{14}C-sarafloxacin base following a single oral 10 mg/kg BW dose was studied in four adult male beagle dogs. Levels of radioactivity in tissues measured at 2 and 6 h after dosing are shown in Table 3 (Volume 4e).

Table 3. Levels (mg equivalents/kg or L) of radioactivity in tissues of male dogs after oral administration of ^{14}C-sarafloxacin base (Dose = 10 mg/kg BW)

Tissue	2 h	6 h	24 h	Tissue	2 h	6 h	24 h
Liver	14	12	2	Bone[2]	3	3	2
Kidney	16	14	1	Retina/uvea	15	43	45
Lung	6	5	1	Blood	3	3	0.4
Brain	0.4	0.7	0.3	Bile	154	454	420
Fat[1]	0.6	0.5	0.6	Urine	89	412	188
Muscle[1]	5	6	1				

[1] Percent dose in muscle and fat calculated assuming those tissues represent 46% and 10% of BW, respectively.
[2] Rib including marrow

The bioavailability of an oral dose of 200 mg sarafloxacin base, equal to 19.6 mg/kg BW, was studied in six adult female dogs. Three different dosage forms were administered – suspension, solution or capsule. The bioavailability of the suspension and capsule were similar. Zero to 32 hour mean AUC values for these formulations were 27 and 23 μg·h/mL, respectively. The mean AUC for the solution was 52 μg·h/mL. The author reported results from other bioavailability studies that showed that, compared to an equal IV dose, the bioavailability of an oral 10 mg/kg BW dose of the solution ranged from 58 to 70%. The relationship between dose and bioavailability appears not to be linear or log linear for the capsule formulation. The suspension produced AUC values that were approximately one half of those obtained from solution, however the author states that, in another study, a lower dose of 10 mg/kg BW, the formulations were equivalent. The basis for the formulation differences was not readily apparent. The author also referenced data from a human clinical study in which capsules of the same lot given to dogs were administered. In humans, urine recoveries of sarafloxacin are considered an approximate estimate of absorption since urinary excretion is the predominant route of elimination in humans. The author referenced results from a human clinical study where urine recoveries ranged from 24% at 1.3 mg/kg bw to 10% at 10.4 mg/kg bw, indicating that absorption rates in humans are considerably lower than absorption rates seen in dogs (Volume 4f).

Humans

A single oral dose of 100, 200, 400 or 800 mg sarafloxacin was administered to 22 healthy male volunteers ranging in age from 20-39 years. Blood samples were taken at pre-test and 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 16, 24, 28, 32 and 48 hours after dosing. Urine was collected at hours 0-4, 4-8, 8-12, 12-16, 16-24, 24-32 and 32-48. Compliance with Good Laboratory Practice principles was not required for this study. The quality and design of this study was consistent with current scientific standards.

Plasma drug levels peaked 1.5-4 hours after dosing and declined biphasically, with the terminal phase becoming dominant approximately 12 h postdosing. The means of the individual peak levels for the 100, 200, 400 and 800 mg dose groups were 140, 180, 240 and 350 ng/ml. respectively. The corresponding dose-normalized peak levels were 106, 62, 44 and 34 ng/mL per mg/kg. Dose normalized AUC values for the 100, 200, 400 and 800 mg groups averaged 860, 570, 410 and 350 ng·h/mL per mg/kg, respectively. The declines in the dose normalized AUC and peak level values as a function of dose provide evidence that the efficiency of absorption deceased by a factor of about 3 as the dose was increased. Terminal phase half-lives averaged 9, 9, 10 and 11 hours for the 100, 200, 400 and 800 mg dose groups, respectively. The major route of elimination was renal excretion of unchanged drug. Renal clearances for the 100, 200, 400 and 800 mg groups averaged 280, 290, 290 and 260 mL/min, respectively. Intergroup variations were not significantly different. Urinary recoveries of unchanged drug averaged 19, 14, 10 and 7% of the administered 100, 200, 400 and 800 mg doses, respectively. The extent of absorption of sarafloxacin decreased from approximately 27 to 34% for the 100 mg dose to 11-13% for the 800 mg dose (Volume 47).

Food Animals

Chickens and Turkeys

Chickens and turkeys were administered ^{14}C-Sarafloxacin hydrochloride by gavage 4 times daily for 5 days. More than 79 - 89% of the dose was excreted within 6 h of dosing (Volume 57, Volume 59).

Metabolism

Mice and Rabbits

The biotransformation of sarafloxacin was investigated in the excreta of mice and rabbits following oral and IV administration in the studies described above (Volume 4a & 4c). In both species parent drug was the main component accounting for more than 80% of the administered dose. Sarafloxacin glucuronide was found as a minor residue (1 - 10% of dose) and N-acetyl-sarafloxacin, 3'-oxo-sarafloxacin- and two unknown compounds were isolated but were less than 1% of the dose.

Humans

The pharmacokinetics and metabolism of sarafloxacin in humans was studied. In this study, 2 groups of 6 volunteers were administered a single oral dose of 100 or 200 mg sarafloxacin and 2 groups of 5 volunteers were administered a single oral dose of 400 or 800 mg sarafloxacin.

The metabolism of sarafloxacin appears to mainly involve oxidative degradation of the piperazinyl substituent, first producing 3'-oxo-sarafloxacin (M3). Subsequent oxidation produces an ethylene diamine substituted quinolone (M5), which in turn is oxidized to an aminoquinolone (M4). The plasma level profiles of M5 parallel those of parent drug, however the AUC of M5 consistently averaged only about 6% of the AUC of sarafloxacin. The concentration of M4 in plasma and urine was considerably lower than that of M5. Due to its weak fluorescence, M3 was not detected in plasma.

In urine, the major drug related peak was sarafloxacin accounting for 75% to 80% of total urinary metabolites. Next to sarafloxacin the most predominant metabolite in urine was tentatively identified as M3. Its levels were typically 1/3 to 1/4 of the corresponding levels of sarafloxacin. Total urinary recoveries of parent drug plus metabolites were low and dose-dependent, changing from 2% to 10% as the dose increased from 100 to 800 mg. The extent of the decrease was similar to the decrease in the dose-normalized AUC. Collectively, M4, M5 and their conjugates accounted for less than 7% of the urinary pattern (Volume 47).

Chickens

Three male and three female chickens were administered 3.34 ± 0.26 mg/kg BW/day ^{14}C-Sarafloxacin HCl (34.4 μCi/mg) by gavage for 5 days. Livers were collected at 6 h after dosing and pooled for each sex. The liver samples were extracted with acidic and basic acetonitrile; 87% (female) and 85% (male) of the residues were extractable. The metabolic profiles were similar for male and female chicken extracts. The metabolites identified are shown in Table 4. This study was performed to GLP (Volume 57).

Turkeys

Three male and three female turkeys were administered 6.9 mg/kg BW/day by gavage ^{14}C-Sarafloxacin HCl (33.3 μCi/mg) for 5 days. Livers were collected at 6 h after dosing and pooled for each sex. The liver samples were extracted with acidic and basic acetonitrile; 83% of the residues were extractable. The metabolic profiles were similar for male and female turkey extracts. The metabolites identified are shown in Table 4.

Table 4. Metabolites of sarafloxacin in poultry liver

Component	% Total Residues in Turkeys		% Total Residues in Chickens	
	Male	Female	Male	Female
Sarafloxacin	20	21	69	65
Sarafloxacin sulphamic acid	7	6	8	13
Sarafloxacin glucuronide	20	25	8	13
Sarafloxacin sulphamic glucuronide	30	16	8	13
Others (4)	6	15	8	9

The main route of metabolism in poultry liver is the formation of either or both a sulphamic acid conjugate at the N-position in the piperazine ring or a glucuronide with the -COOH group. The unidentified minor metabolites were also conjugates because acid or base hydrolysis of the metabolites yielded parent Sarafloxacin. More conjugates were present in the turkey liver than chicken liver.

TISSUE RESIDUE DEPLETION STUDIES

Radiolabeled Residue Depletion Studies

All the studies examined the residues in equal numbers of both male and female birds. The analysis of the results indicated that there were no significant differences in the values for males or females; therefore the results for both sexes were combined.

Chickens

Chickens were administered 0.54 mg/kg BW ^{14}C-sarafloxacin hydrochloride by gavage four times daily for 5 days (Volume 57). The total dose per day was 2.2 mg per bird (3.4 mg/kg BW/day) which simulated 85% of the dose proposed for field use of the drug in drinking water (20 ppm). Groups of six birds were sacrificed at 6, 18, 36 and 72 h

after drug withdrawal. Light muscle, dark muscle, liver, skin with adhering fat, fat and kidney samples were collected and the concentrations of the total radioactive residues (as sarafloxacin equivalents) measured by sample combustion and/or scintillation counting (Volume 58). The results are shown in Table 5.
The residues were highest and most persistent in the liver tissue (Note; kidney not investigated). After one day of drug withdrawal the residues were only measurable in liver and skin. Three days after withdrawal no residues were detected in any of the tissues.

Table 5. Total residues, expressed as µg/kg equivalents of ^{14}C-sarafloxacin-hydrochloride in broilers after oral dosing with 3.4 mg/kg BW ^{14}C-sarafloxacin-hydrochloride per day for 5 days.

Tissue	6 h		18 h		36 h		72 h
	Range	Mean (SD)	Range	Mean (SD)	Range	Mean (SD)	
Liver	221 - 482	322 ± 92	21 - 219	70 ± 75	17 - 28	21 ± 4	<LOD
Skin + Fat	19 - 39	29 ± 7	<LOD(4)-48	26 ± 11*	<LOD		<LOD
Fat	8 - 65	22 ± 21	<LOD		<LOD		<LOD
Light Muscle	24 - 45	35 ± 8	<LOD		<LOD		<LOD
Dark Muscle	18 - 38	28 ± 8	<LOD		<LOD		<LOD
Kidney	NM		NM		NM		NM

* LOD = 21 µg/kg used for determination of SD; NM = not measured.
LOD for light muscle = 5 µg/kg at 6 h, 22 µg/kg at 18, 36 and 72 h. LOD for dark muscle = 6 µg/kg at 6 h, 22 µg/kg at 18, 36 and 72 h. LOD for liver = 4 µg/kg at 6 h, 15 µg/kg at 18, 36 and 72 h. LOD for fat = 6 µg/kg at 6 h, 22 µg/kg at 18, 36 and 72 h. LOD for skin + fat = 5 µg/kg at 6 h, 21 µg/kg at 18, 36 and 72 h.

Turkeys

Turkeys weighing about 2.7 - 3.7 kg were administered 4.25 mg by gavage ^{14}C-sarafloxacin HCl four times daily for 5 days (Volume 59). The total dose per day was 21 mg per bird (ca. 7 mg/kg BW/day), which is higher than the recommended field dose of 4 mg/kg/day (30 ppm in drinking water) for turkeys of a similar age. Groups of six birds were sacrificed at 6, 18, 36 and 72 h after drug withdrawal. Light muscle, dark muscle, liver, skin with adhering fat, fat and kidney samples were collected and the concentrations of the total radioactive residues (as sarafloxacin equivalents) measured by sample combustion and/or scintillation counting. The results are shown in table 6.

Table 6. Total residues expressed as µg/kg equivalents of ^{14}C-sarafloxacin-hydrochloride in turkeys after oral dosing with 7 mg/kg/day ^{14}C-sarafloxacin-hydrochloride for 5 days.

Tissue	6 h		18 h		36 h		72 h	
	Range	Mean#	Range	Mean#	Range	Mean#	Range	Mean#
Liver	181 - 663	388 ± 175	65 - 108	87 ± 20	48 - 80	60 ± 11	25 - 43	35 ± 6
Fat	17 - 165	52 ± 56	<LOD(4)-33	27 ± 3*	<LOD		<LOD	
Skin + Fat	22 - 35	28 ± 5	<LOD(1)-28	22 ± 4*	<LOD(3)-26	19 ± 4*	<LOD(1) - 28	20 ± 5
Light Muscle	6 - 18	12 ± 3	<LOD		<LOD		<LOD	
Dark Muscle	6 - 14	12 ± 4	<LOD		<LOD		<LOD	
Kidney	NM		NM		NM		NM	

± standard deviation (SD) * LOD used for determination of SD. NM = not measured.
LOD for light muscle = 3 µg/kg at 6 h, 13 µg/kg at 18, 36 and 72 h. LOD for dark muscle = 3 µg/kg at 6 h, 12 µg/kg at 18, 36 and 72 h. LOD for liver = 3 µg/kg at 6 h, 15 µg/kg at 18, 36 and 72 h. LOD for fat = 6 µg/kg at 6 h, 25 µg/kg at 18, 36 and 72 h. LOD for skin + fat = 4 µg/kg at 6h, 16 µg/kg at 18, 36 and 72 h.

The residues were highest and most persistent in the liver tissue (Note; kidney not investigated). After 36 hours of drug withdrawal the residues were only measurable in liver and skin. Three days after withdrawal residues were still detected in all the liver samples and in 5 out of 6 skin tissues.

Residue Depletion Studies with Unlabelled Drug

Chickens

Broiler chickens weighing 1.84 -2.54 kg were given sarafloxacin in their drinking water for 119 hours at a concentration of 15.5 - 18.0 ppm (equiv. to 2.7 mg/kg BW / day). Groups of six birds were sacrificed at 0, 26, 96 and 122 h after drug withdrawal (Volume 51). Muscle, liver, lung, skin, fat and kidney samples were collected and the concentrations of sarafloxacin measured by HPLC (Volume 60b). The results are shown in Table 7. The mean values for males were higher than those for females for muscle, liver and kidney at time 0 h but there is no significant difference between the means. Thus the results for both sexes are combined.

Table 7. Residues (μg/kg) in broilers after administration of sarafloxacin at 15.5 – 18.0 mg/l in the drinking water for 119 h.

Tissue	0 h		26 h		96 h		122 h	
	Range	Mean ±SD	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD
Skin	35 - 62	44 ± 13	16 - 26	19 ± 4.3	4.9 - 11.1	7.8 ± 2.5	5.6 - 12.9	8.7 ± 2.8
Muscle	21 - 62	36 ± 16	<LOD		<LOD		NM	
Liver	191 - 929	483 ± 250	5 - 7.5	6.2 ± 0.9	<LOD (5) - <LOQ (1)		NM	
Kidney	112 - 550	229 ± 160	<LOD (4) - <LOQ (2)		<LOD (5) - <LOQ (1)		NM	
Fat	<LOD		<LOD		<LOD		NM	

Values are the range for 6 birds with the mean ± SD; LOD is 2.5 μg/kg and LOQ is 5 μg/kg; NM = not measured.

The residues of parent drug were highest in liver and kidney tissues at zero withdrawal time. The concentration of parent drug fell very rapidly and strongly indicates that this compound is a minor component of the total residues (TR); e.g. (see Table 5) in liver tissues at 18 h mean total residues (TR) = 70 μg/kg, at 36 h mean TR = 21 μg/kg, whereas at 26 h mean sarafloxacin = 6 μg/kg which is probably <20% of TR. In the metabolism study (see above in Table 4) Sarafloxacin formed 65 - 69% of the TR at 6 h. No comparable data was available for kidney but it is most likely that parent drug becomes a minor component of TR at 26 h after drug withdrawal.

The residues of sarafloxacin persisted in the skin. The levels (<13 μg/kg) were low and were not in conflict with the absence of residues in radiodepletion study since they are below the sensitivity of the radio-method (21 μg/kg).

Turkeys

Turkeys weighing 6 - 8.7 kg were given sarafloxacin in their drinking water for 120 hours at a concentration of 21.1 - 28.5 mg/L (equiv. to 2.88 mg/kg BW/day). Groups of six birds were sacrificed at 0, 24 and 120 h after drug withdrawal (Volume 52). Muscle, liver, lung, skin, fat and kidney samples were collected and the concentrations of sarafloxacin measured by HPLC (Volume 60). The results are shown in Table 8.

The residues of parent drug were highest in skin tissues at zero withdrawal time. The concentration of parent drug in liver was low relative to the concentration of TR and strongly indicates that sarafloxacin is a minor component of the total residues, probably about 20% of TR. No comparable data was available for kidney but it is most likely that parent drug is a minor component of TR.

The residues of sarafloxacin persisted in the skin in line with those observed in the radiodepletion study. The levels in both skin and muscle suggest that the parent drug is the major component of residues in these tissues.

Table 8. Residues (μg/kg) in turkeys after administration of sarafloxacin at 21 - 29 ppm in the drinking water for 120 h.

Tissue	0 h		24 h		120 h	
	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD
Skin	35 - 62	44 ± 13	16 - 26	19 ± 4.3	4.9 - 11.1	7.8 ± 2.5
Liver	18 - 54	34 ± 16	3 - 7.8	4.5 ± 1.7*	<LOD	
Muscle	4.2 - 5.9	5.3 ± 0.7*	<LOD		<LOD	
Kidney	6 - 19	12 ± 5	<LOD		<LOD	
Fat	<LOD		<LOD		<LOD	

Values are the range for 6 birds with the mean ± SD. LOD is 2.5 μg/kg and LOQ is 5 μg/kg
*Some values were <LOQ but >LOD.

Bound Residues/Bioavailability.

Chicken and turkey liver samples were extracted with acidic and basic acetonitrile and 13% - 18% were non-extractable (Volume 57 & 59). Neither the identity nor the antimicrobial activity of the bound residues was investigated.

METHODS OF ANALYSIS FOR RESIDUES IN TISSUES

The analysis of residues of sarafloxacin is fully documented for edible poultry tissues (Volume 49, Volume 60, Volume 61a, 61b, 61c, 61d). These methods specifically measure the free sarafloxacin but not the sarafloxacin conjugates. The methods consist of extracting homogenised tissue (muscle, liver, kidney and skin with adhering fat) with acetonitrile or in the case of fat with a mixture of acetonitrile and dichloromethane. After drying, the extract is dissolved in the mobile phase (water + phosphoric acid + tetramethylammonium chloride/acetonitrile/N,N-dimethylformamide) and analysed using HPLC with fluorescent detection. The characteristics of the method are detailed for both broilers and turkeys.

The linear range was 5 - 4000 μg/kg and the LOQ for all poultry tissues was claimed as 5 μg/kg. In liver tissue the CV for a concentration of 5 μg/kg was 14% in broilers and 11% in turkeys. The recoveries for poultry muscle, liver, kidney and skin with adhering fat ranged between 57 and 67 % with CVs 1.2 - 15.2%. The recoveries from fat were 93% for broilers and 100% for turkeys. The method has not been tested in a collaborative study with other laboratories but good reproducibility was achieved in the sponsor's laboratory when three different persons used four different HPLC columns of the same type. There was no interference in the chromatograms from admixing monensin, narasin, salinomycin, flumequine, enrofloxacin, difloxacin and danofloxacin (see Volume 49). Interference from the matrix was < 5 μg/kg. The method was suitable for the routine analysis of large numbers of samples per day.

Unfortunately the above method does not measure the residues which are present as sarafloxacin conjugates (see Table 4). They form the majority of the total residues in turkey liver (47 - 57%) but only 8 - 13% of TR in chicken livers. The sponsors have studied the hydrolysis of the conjugates (see summary in Table 9) and each type of conjugate required specific hydrolysis (Volume 59).

Table 9. Effects of different hydrolysis procedures on the stability of Sarafloxacin conjugates.

Residue	Acid Hydrolysis	Alkaline Hydrolysis	Enzyme Hydrolysis
Parent Sarafloxacin (SFX)	No effect	No effect	No effect
SFX-sulfamic acid	Deconjugation	No effect	Not studied
SFX-glucuronide	No effect (?)	No effect (?)	Deconjugation
SFX-sulfamic acid-glucuronide	Deconjugation of sulfamic acid	???	Deconjugation of glucuronide

The hydrolysis appeared to quantitatively release the sulfamic acid but no values were given for the deconjugation performance for the enzyme hydrolysis (Volume 59).

APPRAISAL

Sarafloxacin is a fluoroquinolone antibiotic for use in broiler chickens and turkeys. The dose for chickens from 3 weeks of age is 20 mg/L in water equivalent to 4 mg/kg body weight and for turkeys from 7 weeks of age the dose is 30 mg/L in water equivalent to 4 mg/kg body weight.

The drug is readily absorbed and rapidly cleared by rats, mice, dogs, rabbits, chickens and turkeys. When chickens and turkeys were administered ^{14}C-sarafloxacin hydrochloride by gavage 4 times daily for 5 days, more than 79 - 89% of the dose was excreted in the first six hours post dosing.

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The metabolism of ^{14}C-sarafloxacin was studied in chickens and turkeys. No differences in metabolism were observed between males and females but the metabolic profiles in the liver were different between the two species. The main route of metabolism in poultry liver is the formation of either or both a sulphamic acid conjugate at the N-position in the piperazine ring or a glucuronide with the -COOH group. The unidentified minor metabolites were also conjugates because acid or alkaline hydrolysis of the metabolites yielded parent sarafloxacin. More conjugates were present in the turkey liver (47 - 57%) than chicken liver (8 - 13%). Parent drug formed 21% residues in turkey liver and 67% in chicken liver.

Residue Depletion

All the studies examined the residues in equal numbers of both male and female birds. The analysis of the results indicated that there were no significant differences in the values for males or females; therefore the results for both sexes were combined.

Chickens (Broilers). Male and female chickens were administered 0.54 mg/kg BW ^{14}C-sarafloxacin hydrochloride by gavage four times daily for 5 days. The total dose per day was 2.2 mg per bird (3.4 mg/kg BW/day) which represented 85% of the dose proposed for field use of the drug in drinking water (20 mg/L). Groups of six birds were sacrificed at 6, 18, 36 and 72 h after drug withdrawal. Light muscle, dark muscle, liver, skin with adhering fat and fat samples were collected and the concentrations of the total radioactive residues (as sarafloxacin equivalents) measured by sample combustion and/or scintillation counting. There was no significant difference between the means for both sexes. At 6 h post-dosing the residues in µg/kg sarafloxacin equivalents were: light muscle, 35 ± 8; dark muscle, 28 ± 8; liver, 322 ± 92; Fat, 22 ± 21; skin with fat, 29 ±7. The residues were below the LOD of 22 µg/kg in muscle and fat at later time points. In skin plus fat the residues were 26 ± 11 µg/kg at 18h and less than the LOD (21 µg/kg) at 36h and 72h. In liver the residues were 70 ± 75 µg/kg at 18h, 21 ± 4 µg/kg at 36h and <LOD (15 µg/kg) at 72h. Thus the residues were highest and most persistent in the liver tissue. After one day of drug withdrawal the residues were only measurable in liver and skin. Three days after withdrawal no residues were detected in any of the tissues.

Broilers weighing 1.84 -2.54 kg were given sarafloxacin in their drinking water for 119 hours at a concentration of 15.5 - 18.0 mg/L (equiv. to 2.7 mg/kg BW / day). Groups of six birds were sacrificed at 0, 26, 96 and 122 h after drug withdrawal. Muscle, liver, skin, fat and kidney samples were collected and the concentrations of sarafloxacin measured by HPLC. The mean residues in µg/kg of parent drug were highest in liver, 483 at 0 h, 6 at 26 h and <LOD (2.5 µg/kg) at 96 h. In skin with adhering fat the residues were present at all times. Mean concentrations in skin with adhering fat in µg/kg at 0 h were 44; at 26 h, 19; at 96 h, 8 and at 122 h. The residues were not found in fat and only found at the zero time point for muscle at a mean residue concentration of 36 µg/kg and in kidney, 229 µg/kg.

Turkeys. Turkeys weighing about 2.7 - 3.7 kg were administered 4.25 mg by gavage ^{14}C-sarafloxacin hydrochloride four times daily for five days. The total dose per day was 21 mg per bird (approximately 7 mg/kg BW/day), which is higher than the recommended field dose of 4 mg/kg/day (30 mg/L in drinking water) for turkeys of a similar age. Groups of six birds were slaughtered at 6, 18, 36 and 72 h after drug withdrawal.

Light muscle, dark muscle, liver, skin with adhering tat, fat and kidney samples were collected and the concentrations of the total radioactive residues (as sarafloxacin equivalents) measured by sample combustion and/or scintillation counting. At 6h post treatment the mean residue concentrations in µg/kg sarafloxacin equivalents were: light muscle, 12; dark muscle, 12; liver, 388; fat, 52; skin with fat, 28. The residues were below the limit of detection (LOD = 13 µg/kg) in muscle at 18 h and later time points. In fat the mean residue concentrations were 27 µg/kg at 18 h, and less than the LOD (25 µg/kg) at 36 h and 72 h. In skin plus fat the mean residues were 22 µg/kg at 18 h; 19 µg/kg at 36 h; and 20 µg/kg at 72 h. In liver the mean residues were 87 µg/kg at 18 h; 60 µg/kg at 36 h; and 35 µg/kg at 72 h.

In another study, turkeys weighing 6 - 8,7 kg were given sarafloxacin in their drinking water for 120 hours at a concentration of 21.1 - 28.5 mg/L (equivalent to 2.88 mg/kg BW/day). Groups of six birds were sacrificed at 0, 24 and

Bound Residues/Bioavailability

Chicken and turkey liver samples were extracted with acidic and basic acetonitrile and 13-18% were non-extractable, Neither the identity nor the antimicrobial activity of the bound residues was investigated.

Analytical Method for Sarafloxacin

The analytical methods specifically measure the free sarafloxacin but not the sarafloxacin conjugates, which form the majority of the total residues in turkey liver (47 - 57%) but only 8 - 13% of the total residues in chicken livers. The methods consist of extracting homogenised tissue (muscle, liver, kidney and skin with adhering fat) with an organic solvent, drying, dissolving the extract in the mobile phase and analysis using HPLC with fluorescent detection. The characteristics of the method are detailed for both broilers and turkeys. The linear range was 5 - 4000 μg/kg and the LOQ for all poultry tissues was claimed as 5 μg/kg. The CVs were determined for each tissue from six replicates at six concentrations over the concentration range noted above. In liver tissue the CV for a concentration of 5 μg/kg was 14% in broilers and 11% in turkeys. The recoveries for poultry muscle, liver, kidney and skin with adhering fat ranged between 57 and 67 % with CVs 1.2 - 15.2%. The recoveries from fat were 93% for broilers and 100% for turkeys. The method has not been tested in a collaborative study with other laboratories but good reproducibility was achieved in the sponsor's laboratory when three different persons used four different HPLC columns of the same type. There was no interference in the chromatograms from admixing monensin, narasin, salinomycin, flumequine, enrofloxacin, difloxacin and danofloxacin. Interference from the matrix was less than 5 μg/kg. The method was suitable for the routine analysis of large numbers of samples per day.

Choice of Marker Residue

Parent drug is the clear choice as marker residue (MR) because it is the major residue in all tissues. It is neither necessary nor possible to correlate the values observed for the total residues with those found for the parent drug in the unlabeled residue study. This is because the sponsors have shown that the microbial activities of some of the metabolites (N-acetyl-sarafloxacin, N-formyl-sarafloxacin, 3'-oxo-sarafloxacin and the sulfamic acid conjugate of sarafloxacin) are significantly lower than that of sarafloxacin.

Choice of Target Tissues

The residues in poultry are highest in liver and kidney and persist in skin with adhering fat. The kidney is not normally a target tissue for poultry and therefore the main target tissues should be liver and skin with adhering fat (skin and fat). Because muscle is the major edible tissue for poultry and residues are found in this tissue at short withdrawal times an MRL is set for muscle.

Maximum Residue Limits

The ADI for Sarafloxacin is 0 - 0.3 μg/kg body weight. This permits daily 18 μg per 60 kg person. The following factors are used to set MRLs for chickens and turkeys.

1. Sarafloxacin is the marker residue.
2. The microbiological activity of residues other than parent drug is significantly lower.
3. The LOQ for the analytical methods are 5 μg/kg.
4. As no residues are detectable in poultry muscle at 18 h and beyond, the MRL should equal two times the LOQ.
5. The residues in liver and kidney are higher than residues in muscle, skin and fat.
6. The MRL for chickens are equally applied to turkeys

The Committee recommends MRLs f or chickens and turkeys of 10 μg/kg in muscle, 80 μg/kg in liver, 20 μg/kg in fat and of 80 μg/kg for kidney expressed as parent drug. Using these values for the MRLs, the theoretical maximum daily intake is 16 μg.

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