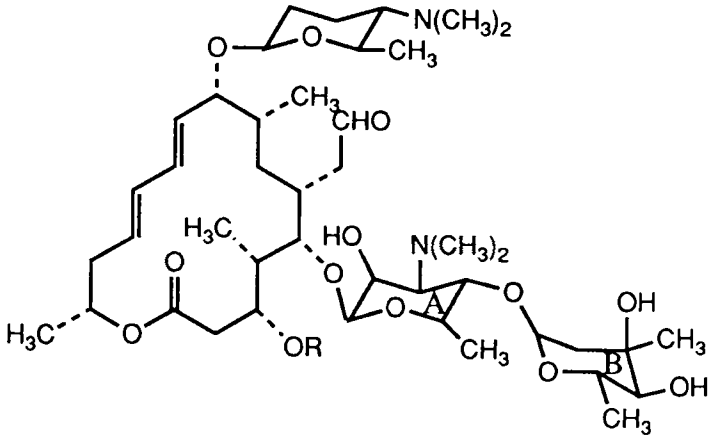
SPIRAMYCIN

IDENTITY

Chemical: Spiramycin

Synonyms: CAS 8025-81-8; Foromacidin; Sequamycin; Selectomycin; Rovamycin; Provamycin

Structural formula:



Molecular formula:

Spiramycin I $(C_{43}H_{73}N_2O_{13})$-O-R : -R = -H

Spiramycin II $(C_{43}H_{73}N_2O_{13})$-O-R : -R = $-COCH_3$

Spiramycin III $(C_{43}H_{73}N_2O_{13})$-O-R : -R = $-COC_2H_5$

Molecular weight:

Spiramycin I	843.07
Spiramycin II	885.11
Spiramycin III	899.14

OTHER INFORMATION ON IDENTITY AND PROPERTIES

Pure active ingredient:

Appearance:	White or faintly yellowish powder
Melting point:	128 - 137 °
Solubility:	Barely soluble in water, freely soluble in acetone, alcohol and methanol, sparingly soluble in ether
Optical rotation:	[a]S(20,D) = -80° (c = 1 in methanol)
UV_{max}:	231 nm (ethanol)
pH:	8.5 - 10.5 (0.5% solution)

Related compounds:	Spiramycin II	≤ 5.0 %
	Spiramycin III	≤ 10.0 %
Other compounds:		≤ 2.0 %
Microbiologic potency (min)		3200 IU/mg

(Budavari, 1989; Pascal, 1986)

RESIDUES IN FOOD AND THEIR EVALUATION

CONDITIONS OF USE

General

Spiramycin is a macrolide antibiotic produced by certain strains of *Streptomyces ambofaciens*. It is extracted from culture media and then concentrated, filtered and purified. Spiramycin is a mixture of three components named Spiramycin I, II and III. Spiramycins II and III are, respectively, the monoacetic and monopropionic esters in position 4 of the macrocyclic lactone ring of Spiramycin I, as shown above. Spiramycin exhibits bacteriostatic activity against staphylococci, aerobic and anaerobic streptococci, corynebacteria, clostridia as well as mycoplasma, rickettsia and *Toxoplasma gondii.* It is practically without activity against *Escherichia coli* and *Pseudomonas aeruginosa.* Spiramycin induces a bacteriopause characterized by persistence of bacteriostasis after removal of the antibiotic. The mechanism of action of spiramycin, as with other macrolide antibiotics, is via competitive binding at the 50 S ribosome and inhibition of bacterial protein synthesis. Spiramycin is indicated for the treatment and prevention of pneumopathies, enteropathies, mastitis, metritis, omphalitis, omphalophlebitis, arthritis and foot-rot in cattle and, in pigs, for the treatment and prevention of pneumopathies, pig-house cough, infectious gastroenteritis, swine erysipelas, mastitis, prevention of neonatal infections in piglets and atrophic rhinitis.

Dosage

The following is provided for product and dosing information for the use of spiramycin in cattle and swine.

For use in cattle, the following two water soluble products are provided as the adipic acid salts.

	SUANOVIL 20®	SUANOVIL L.A.®
Spiramycin	60×10^6 IU	100×10^6 IU
Monomethylacetamide	49 g	49 g
Benzyl alcohol	4.16 g	1 g
Water for injection	100 ml	100 ml

1 IU is approximately = 0.3 μg of spiramycin base expressed in relation to the WHO standard titering 3200 IU/mg.

The dosing regimen for young cattle is 100 000 IU/kg (10 ml/100 kg of Suanovil L.A.®) twice at 48 hour intervals and for metaphylaxis, a single injection of 100 000 IU/kg.; for

calves, 75 000 IU/kg (5 ml/40 kg of Suanovil 20 ®) and for adult cattle (mastitis and foot-rot) 30 000 IU/kg (5 ml/100 kg of Suanovil 20 ®). The sponsor recommends a dose of 75 000 IU/kg for other species. (Suanovil 20 and Suanovil L.A. are registered trademarks of RHONE MERIEUX).

Spiramycin for use in animal feed, primarily for prevention and treatment of Enzootic Pneumonia in swine, is provided in the form of a salt of embonic acid and is sometimes referred to as the embonate form. Spiramycin embonate is available as two different premixes: SPIRAMIX 200® and SPIRALAC 200®. The formulations are as follows:

SPIRAMIX 200®
Spiramycin (embonate)..... 64 MIU
Corn meal.....q.s.p. 100 g

SPIRALAC 200®
Spiramycin (embonate)..... 64 MIU
Lactose.....q.s.p. 100 g

The antibiotic is incorporated in feed at a rate of 200 to 400 ppm as spiramycin embonate. Depending on the rate of feed intake which varies with the weight of the animal, the corresponding dose to pigs will be 30 000 IU/kg/d to 50 000 IU/kg/d (i.e. 9.5 mg/kg/d to 16 mg/kg/d) for 7 days. (SPIRAMIX 200 and SPIRALAC 200 are registered trademarks of RHONE POULENC ANIMAL NUTRITION).

METABOLISM

In data submitted, the statement is made that spiramycin can be converted into neospiramycin (structure not given) in the tissues and particularly in the liver. It is further stated that neospiramycin exhibits similar antibacterial activity to spiramycin. The reference is to a nonpublic document that has no number, date or author.

TISSUE RESIDUE DEPLETION STUDIES

Radiolabeled Residue Depletion Studies

No radiolabel residue depletions studies were submitted by the sponsor.

Residue Depletion Studies with Unlabeled Drug

Cattle

Eighteen young bovines, 6 males and 12 females, were used in the study. The animals received two injections of Suanovil 20 by intramuscular injection at 100,000 IU/kg BW at 48 hour intervals. The animal ranged in weight from 177 to 350 kg and included both sexes. Groups of three animals were sacrificed at 14, 21, 28, 35, 42 and 49 days after the final administration. Only males were sacrificed at post treatment days 14 and 21 while only females were slaughtered on days 28, 35, 42, and 49. Most (12) of the animals were of the Charolais breed or cross with four Limousin, a Normand and a Pie Rouge completing the animal breeds.

Spiramycin was determined in samples collected from the liver, kidney, muscle and injection site at all sacrifice times. Fat was assayed for spiramycin beginning on day 28 and heart was sampled beginning on day 35. Both spiramycin and neospiramycin concentrations in the tissues were determined using high-performance liquid chromatography. Mean recovery of spiramycin was 75.5, 84.7 and 97% from muscle, liver and kidney, respectively. Mean recovery of neospiramycin was 82.8, 72.0 and 80.3%. The limit of detection was estimated to be 0.05 IU/g, with quantification limits of 0.1 IU/g in muscle and liver and 0.2 IU/g in liver for both spiramycin and neospiramycin. The results are shown in Table I. Neospiramycin concentrations were expressed in spiramycin equivalents by applying a correction factor of 0.88 which corresponds to the ratio between neospiramycin titre (4100 IU/mg) and the spiramycin titre (4656 IU/mg). The total concentration of spiramycin was then expressed in μg/g of spiramycin in relation to the 1962 WHO standard titering 3200 IU/mg (Sanders and Delepine, 1990 a).

Table I. Concentration of Spiramycin (μg/g) in muscle, injection sites, liver, kidney and fat (mean ± standard deviation).

	Concentration of Spiramycin (μg/g)				
Time	Muscle	Injection Site 1	Injection Site 2	Liver	Kidney	Fat
Day-14	0.09±0.02	20.91±14.05	35.12±14.91	0.48±0.09	0.47±0.15	NS
Day-21	< 0.06	10.23± 6.59	10.30± 4.89	0.30±0.13	0.17±0.05	NS
Day-28	< 0.03	0.47± 0.38	0.60± 0.04	0.14±0.03	0.05±0.02	ND
Day-35	< 0.03	0.11± 0.07	0.31± 0.13	< 0.12	< 0.03	*
Day-42	ND	0.13± 0.06	0.16± 0.12	< 0.12	< 0.03	<0.03
Day-49	ND	0.05± 0.08	0.04± 0.05	< 0.06	ND	ND

ND = Not detected (< 0.015 μg/g)
NS = Not sampled
* = 0.05 ± 0.06

From the edible tissues other than injection site, residues were highest and were quantitated in liver for 28 days after treatment and detected in liver for 49 days. Detectable, although not quantifiable, residues were present in muscle, kidney and fat at days 35, 42 and 42, respectively. Not surprisingly, residues of the antibiotic were highest and persisted longer at the injection sites, although after 35 days, they were below 0.16 ppm. Although not shown here, residues of neospiramycin were approximately the same concentrations as spiramycin.

Residues of spiramycin in milk were determined following intramuscular administration of 30,000 IU/kg BW Suanovil 20 to six dairy cows.

Milk samples were collected before administration of the Suanovil 20 and at each milking following administration for 25 milkings. The spiramycin concentrations in the milk were determined using the microbiological agar diffusion method with *Micrococcus luteus*. The quantification limit was 0.2 IU/ml equivalent to 0.062 μg/ml of spiramycin expressed

in relation to the 1962 WHO standard titering 3200 IU/mg. The mean concentrations are presented in Table II (Moulin & Sanders, 1989 a).

Table II. Concentration of spiramycin in milk (mean ± standard deviation).

Milking	Concentration (μg/ml)
1	16.54 ± 7.50
2	10.00 ± 3.18
3	5.27 ± 1.62
4	2.74 ± 0.90
5	1.50 ± 0.40
6	1.06 ± 0.25
7	0.74 ± 0.15
8	0.57 ± 0.09
9	0.43 ± 0.08
10	0.40 ± 0.05
11	0.32 ± 0.06
12	0.30 ± 0.06
13	0.24 ± 0.10
14	0.19 ± 0.03
15	0.16 ± 0.03
16	0.14 ± 0.03
17	0.09 ± -
18 - 25	ND

ND = Not detected (< 0.06 μg/ml)

Forty-two female Friesian/Holstein calves, aged 8-15 days, were used in a study. Calves were acclimated for one week. The animals were divided into three groups. Group 1 (4 calves) served as control animals. Group 2 (22 animals) recieved 25 mg spiramycin per kg body weight daily for seven days, in the feed. Group 3 (16 animals) were treated with spiramycin (25 mg/kg BW) plus oxytetracycline (40 mg/kg BW), in the feed, for seven days. Samples were collected and assayed microbiologically (Pascal et al., 1990a). The results are presented in Table III.

Table III. Spyramycin Tissue Concentrations (μg/g) in Calves Recieving Spiramycin, 25 mg/kg BW per day, for 7 consecutive days, Alone or in Combination with Oxytetracyline, 40 mg/kg BW per day

Days Post-Treatment	Liver	Kidney	Muscle	Fat
3	9.17	13.7	0.2	0.15
7	3.03	5.3	<0.1	<0.1
14	1.1	1.7	<0.1	<0.1
24	0.2	<0.1	<0.1	ND
35	<0.1	ND	ND	ND

ND = Not detected

Swine

Fifteen pigs, 10-12 weeks of age, recieved 25 mg spiramycin per kg BW per day intramuscularly for three consecutive days. Three animals were slaughtereed at each sacrifice time. Tissue samples were collected from liver, kidney, muscle, fat, skin, heart and brain. Samples were analyzed using a microbiological assay with *Micrococcus luteus* (May and Baker, 1967). The results are summarized in Table IV.

Table IV. Spiramycin Tissue Concentrations (μg/g) in Pigs Recieving Intramuscular Spiramycink, 25 mg/kg BW per day for 3 consecutive days

Days Post-Treatment	Liver	Kidney	Muscle	Fat	Skin	Heart	Brain
1	9.01	21.59	0.29	0.03	0.11	0.32	0.04
3	2.26	4.75	0.20	0.09	0.20	0.20	*
5	0.68	1.24	0.05	0.05	0.07	0.10	*
7	0.49	0.48	*	0.04	0.04	0.07	*
14	*	0.04	*	*	*	*	*

* = Less than the assay sensitivity of 0.025 μg/g

A series of residue studies were conducted in 25 to 30 kg male and female Large White X Landrace pigs to determine the tissue residues of spiramycin following oral administration of spiramycin embonate. In each study, three animals were slaughtered at each sacrifice time. The dose is indicated in each table. Analysis of spiramycin and its active metabolites in tissues was conducted using the agar diffusion method with *Sarcina lutea* ATCC 9341 as the test organism. Results are summarized in Tables V and VI and are relative to the WHO standard titrating 3200 IU/mg (Pascal et al., 1990b).

Table V. Spiramycin Tissue Concentrations in Pigs Receiving spiramycin 16 mg/kg/day for 7 days (μg/g)

Days Post-Treatment	Liver	Kidney	Muscle	Fat
0.5	6.26 ± 1.26	8.90 ± 1.75	0.12 ± 0.01	< 0.10
3	1.44 ± 0.24	1.30 ± 0.56	< 0.10	< 0.10
7	0.58 ± 0.19	0.23 ± 0.07	< 0.10	< 0.10
10	< 0.30	< 0.15	*	< 0.10
15	< 0.30	< 0.15	*	*
20	< 0.30	< 0.15	*	*

* = not determined

Table VI. Spiramycin Tissue Concentrations in Pigs Receiving Spiramycin, 25 mg/kg/day for 7 days (μg/g)

Days Post-Treatment	Liver	Kidney	Muscle	Fat
7	1.45 ± 0.40	0.56 ± 0.11	< 0.10	< 0.10
10	0.89 ± 0.35	0.19 ± 0.05	*	< 0.10
20	< 0.30	< 0.15	*	*

* = not determined

Excretion and tissue concentration of spiramycin were studied in the pig following administration by the oral (4 animals) or the subcutaneous (10 animals) route. The results are summarized below in Table VII. Sample concentrations are the average of two pigs per sacrifice time (Ferriot and Videau, 1971).

Table VII. Spiramycin Concentrations in the Tissues of Pigs Treated with Oral and Subcutaneous Spiramycin

Route of Adm.	Time After Dosing	Dose mg/kg	Concentration of Spiramycin in μg/ml or g						
			Liver	Bile	Kidny	MG[a]	Lung	Int[b]	Muscle
Oral	24 h	50	19.00	37.50	30.00	12.00	10.75	14.00	1.25
	24 h	100	56.25	217.5	67.50	33.75	44.25	62.50	1.85
SC	24 h	12.5	8.25	100.0	29.00	13.50	12.75	4.50	1.65
	24 h	25	19.25	155.0	55.00	24.75	37.50	13.25	0
	24 h	50	52.50	345.0	112.5	42.50	81.25	50.00	6.0
	48 h	50	27.50	67.50	77.50	147.5	22.50	13.75	0
	8 d	50	18.25	-	-	-	-	-	-

[a] MG = Mesenteric ganglion
[b] Int = Intestine

As expected, the parenteral dose proved to have significantly greater bioavailability than the oral route at an equivalent dose (50 mg/kg, at 24 h). However, the relatively high levels of spiramycin residues in organs and bile by the oral route is somewhat surprising. Concentrations in muscle by both routes is much lower than in the other tissues and deplete rapidly by comparison but can be detected at 24 hours after dosing by either route. The concentrations of antibiotic remain at bactericidal or bacteriostatic levels for at least 48 hours post treatment in the case of parenteral dosing. High levels of residue (18 ppm) were detected in liver 8 days after treatment.

Poultry

Thirteen broilers, weighing approximately 1.2 kg each, were treated with 300 ppm spiramycin in the feed for ten days. The average daily intake of spiramycin was calculated to be 43 mg. The birds were slaughtered immediately following removal of the medicated feed and at 1, 3, 5 and 8 days post-treatment. Samples of liver and muscle were analyzed with a microbiological assay using *Micrococcus luteus*, ATCC 9341 (Benazet and coll., 1960). The results are presented in Table VIII.

Table VIII. Spiramycin Tissue Concentrations (μg/g) in Broilers Recieving Spiramycin 300 ppm in the Feed, for 10 consecutive days

Days Post-Treatment	Liver	Muscle
0	3.78	0.19
1	1.67	0.08
3	0.89	0.04
5	0.21	<0.02
8	<0.02	<0.02

Note: The assay sensitivity = 0.02 μg/g

METHODS OF ANALYSIS FOR RESIDUES IN TISSUES

General

Microbiological and high performance liquid chromatographic methods have been employed for the determination of spiramycin concentrations in plasma, serum, tissues and milk.

The microbiological agar diffusion method uses *Micrococcus luteus* ATCC 9341 as the test organism. Serum samples are analyzed following dilution with phosphate buffer, as needed. Agar plates are prepared using Difco #9 medium and seeded with the test organism. Standards are prepared in blank serum. Cylinders are placed on the seeded plates and 200 μl of material, sample or standard, is added. The diffusion continues for 1 hour at ambient temperature and then for 18 hours at 30°. Zones of inhibition are quantitated by extrapolation from standards. The method has a quantitation limit of 0.1≈IU/ml (Huet et al. 1988).

Milk samples are prepared in much the same way as serum samples. Samples may be analyzed directly or following dilution with phosphate buffer. Plates are prepared using Difco #9 medium and are seeded with *Micrococcus luteus*. Samples (250 μl) are placed into the test cylinders. Following diffusion for 0.5 hours at ambient temperature, the plates are incubated for 18 hours at 37°. Zones of microbial inhibition are measured and the concentration of spiramycin in the samples determined by extrapolation to known standards. The quantitation limit in milk is 0.2 IU/ml (Moulin and Sanders, 1989 b).

High performance liquid chromatography (HPLC) has been used to measure spiramycin concentrations in plasma. Test samples and standards prepared in blank plasma are processed through C2 cassettes using 4% acetonitrile. A 5μ reversed phase LiChroCart RP 8 column, 125 mm X 4 mm, with an RP 8 precolumn is used for the separation. Samples are eluted with sulfuric acid, 0.5%, and acetonitrile (79:21, v/v). A UV detector monitors sample elution at 231 nm. The method is linear from 0.2 to 104 IU/ml with average recoveries of approximately 85%. The limit of quantitation in plasma is 0.2 IU/ml (Mourot and Sanders, 1988).

Determination of tissue concentrations of spiramycin and neospiramycin were also determined using reversed phase HPLC. Test tissues and standards prepared in blank tissues are extracted with chloroform and the extracts are processed through cassettes by eluting with chloroform. A 5μ reversed phase LiChroCart RP 8 column, 125 mm X 4 mm, with an RP 8 precolumn is used for the separation. Samples are eluted with 0.5% sulfuric acid and acetonitrile (80:20, v/v). A UV detector monitors sample elution at 231 nm. The method is linear for concentrations between 0.2 and 10 IU/g and recovery of spiramycin from extracted standards is 75.5%, 84.7% and 97% for muscle, liver and kidney, respectively. Recovery of neospiramycin is 82.8%, 72% and 80.3%. The quantitation limit was 0.1 IU/g in muscle and kidney and 0.2 in the liver. The limit of detection was estimated to be 0.05 IU/g (Sanders and Delepine, 1990 b).

APPRAISAL

Residues of spiramycin were highest in cattle liver after intramuscular injection other than injection site with detectable residues (<0.06 ppm) through 49 days after treatment but with quantifiable residues (0.14 ppm) persisting only through day 28. In pigs, a similar situation exists with liver retaining the greatest concentrations of residue. Residues in swine liver deplete to below the limit of quantitation (<0.30 ppm) after 10 to 20 days depending on the oral dose received. However, the situation for residues resulting from injection of spiramycin shows a much different picture with much larger amounts of residue (18 ppm) observed at 8 days after treatment. A residue depletion study extending beyond 8 days for residues resulting from injection of spiramycin in pigs was not submitted for review by the sponsor. However, it is likely that residues may persist as long in swine as they do in cattle for the injectable form of the drug.but there is little indication from the information presented that the injectable form of the drug is likely to be used in market weight pigs. Thus, liver is the target tissue for quantitation of residues of spiramycin in both cattle and swine and the parent compound or microbiologically active residues resulting from its use may serve as the marker residue.

The quantitation of spiramycin residues for more that 8 days (16 milkings) is notable; however, concentrations of the antibiotic were well below 1 ppm after 4 days (8 milkings).

Methods for residue analysis in edible tissues of swine and cattle as well as milk are available for the determination of spiramycin and neospiramycin, although significant information on the latter compound was not available from the sponsor. However, it is claimed that neospiramycin (de*ectable by HPLC) has antibiotic activity with a potency

similar to the parent compound. As mentioned above, the detection of neospiramycin may serve as a partial confirmation that the parent antibiotic has been used but a more definitive confirmatory procedure such as mass spectrometry was not presented. The molecular mass of the antibiotic (843.07 to 899.14) would require relatively sophisticated mass spectrometric instrumentation. A microbiological assay for spiramycin in feeds was adopted by the E.E.C. committee of experts (JOCE of 6.9.84, L 238/34 to 38).

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