SPECTINOMYCIN

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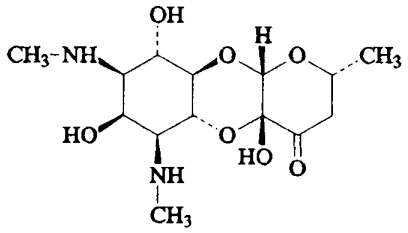
IDENTITY

Chemical name: Decahydro-4a,7,9-trihydroxy-2-methyl-6,8-bis(methylamino)-4H-pyrano[2,3-b][1,4]benzodioxin-4-one

(Also available as dihydrochloride and sulfate)

Synonyms: Actinospectacin; Spectinomycin dihydrochloride pentahydrate, Trobicin, Spectinomycin hydrochloride hydrate, M-141, Stanilo, Spectam, Spectogard, Togamycin, Espechinomicina; Actinospectatin sulfate, Spectinomycin sulfate, Antibiotic 153a

Structural formula:



Molecular formula: $C_{14}H_{24}O_7N_2$

Molecular weight: 332.36

OTHER INFORMATION ON IDENTITY AND PROPERTIES

Pure active ingredient: Spectinomycin

Appearance: Amorphous solid

Melting Point: 184-194°C - Spectinomycin
≈ 185°C - Spectinomycin dihydrochloride pentahydrate
≈ 185°C - Spectinomycin sulfate tetrahydrate

Solubility: **Spectinomycin** - soluble in water, methanol and ethanol. Practically insoluble in acetone and hydrocarbon solvents.
Spectinomycin dihydrochloride pentahydrate - soluble in water, methanol and propylene glycol. Practically insoluble in alcohol, chloroform and ether.
Spectinomycin sulfate tetrahydrate - very, very soluble in benzene, chloroform, ethanol and acetone.

Optical Rotation:	$[\alpha]_D$	Spectinomycin	+7.6°
		Spectinomycin dihydrochloride pentahydrate	+15 to +21°
		Spectinomycin sulfate tetrahydrate	+10 to +14°

RESIDUES IN FOOD AND THEIR EVALUATION

CONDITIONS OF USE

Spectinomycin is an aminocyclitol antibiotic produced by *Streptomyces spectabilis*. It is active against Gram positive and Gram negative organisms, and against mycoplasmas. Anaerobic organisms are mostly resistant to spectinomycin. In human medicine, spectinomycin is used for the treatment of uncomplicated gonorrhea. In veterinary medicine, it is used therapeutically for treatment of bacterial respiratory and enteric infections in cattle, swine, sheep, goats, and poultry.

Dosages

Spectinomycin is administered orally by aqueous solutions, and feed, or by injection, intramuscular or subcutaneous. The typical dose range for spectinomycin administered by injection is 7.5-12.5 mg/kg body weight. Poultry may be administered subcutaneous injections of 1-5 mg spectinomycin/bird. Spectinomycin is also combined with lincomycin in a 1:2 ratio of lincomycin-spectinomycin for injectable formulations and for in-water formulations for poultry. The oral formulation for swine is a 1:1 combination with lincomycin. Calves, sheep, and swine may be dosed with lincomycin-spectinomycin (L1:S2) by intramuscular injection at 15 mg /kg bw. Oral dose levels for lincomycin-spectinomycin (L1:S2) in swine are 5-10 mg/kg bw in water and 11-88 g/ton of feed. The dose level for lincomycin-spectinomycin (L1:S2) in poultry is 30 mg/kg bw for intramuscular injection and 50-150 mg/kg bw via drinking water. Spectinomycin is available as a sulfate tetrahydrate or a dihydrochloride pentahydrate. These form possess identical biological activity.

METABOLISM

Studies on the metabolism, pharmacokinetics, and residues of spectinomycin have been hindered by the inability to radiolabel the molecule and to develop an assay capable of detecting residues in tissues and blood with sufficient sensitivity. Recently, both of these deficiencies have been resolved and studies on pharmacokinetics and residue depletion have been conducted and reported. In this monograph, the results of studies that use validated methods with adequate sensitivity will be reported in full. Only brief summaries of the results of the earlier studies that did not use satisfactory methods will be presented.

Pharmacokinetics and Metabolism in Food and Laboratory Animals

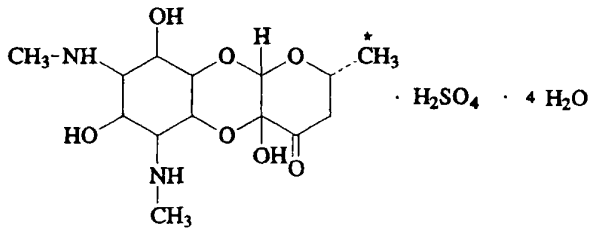
Studies have shown that spectinomycin is only slightly absorbed from the gastrointestinal tract following oral administration. An oral dose of spectinomycin is excreted mostly in the feces. After intramuscular injection, spectinomycin is rapidly absorbed and then excreted primarily in the urine. Repeated administrations do not result in accumulation of drug. Cattle dosed with 10 mg/kg bw spectinomycin a single time by i.m. and i.v. injections, or dosed multiple times produced similar plasma concentrations. The bovine plasma half-life has been measured at about 2 hours. In swine dosed orally with spectinomycin, plasma concentrations were below 30 μg/kg at all times tested. In swine dosed by i.m., spectinomycin plasma concentrations were above 30 μg/kg up to 12 hours postdose. The swine plasma half-life was calculated to be about 0.98 hours.

Many studies were performed with the combination of lincomycin and spectinomycin. It has been shown that the pharmacokinetics and detection of spectinomycin are not influenced by the presence of lincomycin. Therefore in this monograph, data obtained with both spectinomycin and the combination lincomycin-spectinomycin are used in the assessment of spectinomycin.

No efficient way of labeling spectinomycin with ^{14}C has been found. The compound, 4'-^{3}H-spectinomycin, was synthesized, but studies with orally dosed rats demonstrated exchange of the ^{3}H-label. Therefore, a method was developed to synthesize spectinomycin which was labeled with deuterium or tritium on the C6'-methyl side chain

of the pyran ring.

Labeling spectinomycin on the C6'-methyl side chain★ of the pyran ring makes a tritium label that is stable *in vitro*. The stability of the radiolabel was studied *in vivo* with rats and with target species, swine and cattle, treated orally and i.m. It was found that some metabolism to tritiated water occurs *in vivo*. From 2.9 to 3.5% of the ^{3}H-spectinomycin is converted to tritiated water.



Spectinomycin-6'-^{3}H-methyl

Spectinomycin-6'-^{3}H-methyl is considered suitable for use in metabolism studies. Rat and swine studies with ^{3}H-spectinomycin showed a similar disposition of the dose. Spectinomycin is poorly absorbed from the gastrointestinal tract of cattle and swine. Most of the oral dose is excreted in feces while most of the i.m. dose was excreted in urine. In all species tested, the target tissue is the kidney and the major metabolite is spectinomycin. The following studies provide information on tissue residues and the suitability of the label.

Rat

Four Sprague-Dawley rats were dosed orally daily with aqueous solutions of ^{3}H-spectinomycin for up to five days. Urine and feces were collected daily. Tissues were collected 24 hours postdose. Total radioactivity in urine was counted by LSC. Feces and tissues were homogenized, combusted, and total radioactivity counted by LSC. Samples of urine and homogenates of tissue and feces were lyophilized and the resulting tritiated water was trapped and counted by LSC. Presence of tritium in urine and trapped water was confirmed by HPLC with a radioactivity monitor. About 60-80% of the total dose was excreted in the feces and about 2-3% in the urine. Analysis of the water trapped from lyophilized samples showed that in urine, from 23-43%, and in the liver, kidney and muscle from 74-100% of the radioactivity was recovered as tritiated water. These data demonstrate the ^{3}H-spectinomycin labeled at the 4'-position of the pyran ring is biologically unstable and unsuitable for metabolism studies (Hamlow and Jaglan, 1988).

Four Sprague-Dawley rats were dosed orally three times with 5 mg/kg bw ^{3}H-spectinomycin labeled at the 6'-methyl side chain of the pyran ring by the method of Kornis and Hornish (1991). Five groups of Sprague-Dawley rats (2 rats per group) were injected intramuscularly with 5 mg/kg bw/day ^{3}H-spectinomycin for up to 5 days. Urine and feces were collected 24 hours after each dose. Tissue and plasma were collected 24 hours after the last dose. Total residues were measured by combustion. Tritiated water was determined by lyophilizing samples, trapping the tritiated water, and then counting the radioactivity in the tritiated water. Four to seven percent of the total dose was absorbed. An average of 44.6% of the administered oral dose was found in the feces and only 5.4% in the urine. Trace amounts of total residues were present (<0.1 μg/g) in muscle and fat while mean liver residues were 0.12 μg/g. Kidney had the highest residues of ^{3}H-spectinomycin equivalents (0.2-0.6 μg/g). From the intramuscular treatment, 54-73% of the dose was excreted in urine and 1-24% in feces. Tissue residues were low except for kidney (1.8-10.6 μg/g spectinomycin equivalents). The tissue residues in μg/g ^{3}H-spectinomycin equivalents after i.m. injections are shown in Table 1. The mean percent of tritiated water after oral treatment was 70.4% in liver and 73.4% in muscle of the label, respectively. These results may indicate that the methyl group has been metabolized to -CH_2-OH and -CHO while still retaining tritium in the molecule. In kidney, the mean percent of the tritiated water was 37.7% after oral treatment. The remaining label represents spectinomycin and metabolites. The mean percent of tritiated water after i.m.

treatment was 5.3% in kidney, 66.6% in muscle, and 44.9% in liver. After i.m. treatment, the amount of tritiated water/g of water in tissue and fluids varied from 3309 to 3589 DPM. These results indicate that the tritiated water was uniformly distributed throughout the animal. From the results of both oral and i.m. administration, the kidney was demonstrated to be the target tissue. The data indicate that ^{3}H-spectinomycin metabolizes to tritiated water but most of the label is present in the target tissue, kidney. ^{3}H-spectinomycin labeled in the 6-methyl position appears to be useful for metabolism studies (Jaglan et al., 1991a; Roof and Jaglan, 1993).

Table 1. Rat Tissue Residues (μg/g ^{3}H-Spectinomycin Equivalents) 24 Hours after the Last of Multiple i.m. Doses of 5 mg/kg bw ^{3}H-Spectinomycin

Number of Doses	Liver	Kidney	Muscle	Fat
1	0.3	3.1	0.1	<0.1
1	0.4	3.5	0.1	0.1
2	0.5	5.4	0.4	0.2
2	0.6	4.2	0.1	0.2
3	0.7	6.8	0.2	0.2
3	0.6	8.4	0.1	0.1
4	0.2	10.6	0.3	0.1
4	0.2	1.8	0.1	0.1
5	0.3	3.2	0.1	0.1
5	0.3	3.2	0.1	0.1

Dog

Two studies conducted with dogs dosed orally with 100 or 500 mg spectinomycin/kg bw or injected i.m. one time with 40 mg/kg bw, used a less sensitive microbiological method to measure spectinomycin plasma concentrations. After oral dosing, the mean peak serum concentrations with 100 and 500 mg/kg bw doses were 22 μg/ml and 80 μg/ml, respectively, at approximately 4 hours after dosing. The mean half-life value for the 100 mg dose level was 2.72 hours (Stern et al., 1984a). After i.m. injection, the drug was rapidly absorbed with a mean peak serum concentration of 78 μg/ml, a T_{max} of 0.67 hours, and an elimination half-life of 1.15 hours (Stern et al., 1984b).

Cattle

Eight cows were dosed with a single i.v. injection of 20 mg spectinomycin/kg bw. The C_{max} ranged between 52 and 71 μg/ml with an average elimination half-life of about 1.2 hours (Ziv and Sulman, 1973).

The pharmacokinetic data from cows dosed i.m. with spectinomycin (Ziv and Sulman, 1973) were compared to that from calves dosed i.m. with lincomycin-spectinomycin (Gobbi and Quintavalla, 1990), and it was concluded that the pharmacokinetics of spectinomycin are not changed when lincomycin is present (Gilbertson, 1991).

Two Holstein steers (150 kg) were injected i.m. with a single dose of 22 mg ^{3}H-spectinomycin/kg bw. Total daily urine and feces were collected. The animals were slaughtered at 24 and 72 hours post-treatment. Liver, kidney, lungs, muscle and total GI contents were collected. About half of the dose, 55.8% at 24 hours and

46.6% at 72 hours, was excreted in the urine. The percent of radioactivity determined to be tritiated water was 6.7% at 24 hours and 5.8% at 72 hours. When these values are corrected for the tritiated water content of ^{3}H-spectinomycin, the amount becomes 2.9% which is similar to values found with rat and swine (Roof et al., 1993).

Two studies were undertaken in which the pharmacokinetics in cattle were measured using an HPLC assay (LOD 0.1 μg/g) that was developed by Haagsma (1991b). In the first study which was a cross-over design, six nonruminating calves (60-80 kg) were randomly devided into Groups A and B containing 3 animals each. Group A received a single i.v. injection and Group B a single i.m. injection of 10 mg spectinomycin/kg bw (15 mg lincomycin-spectinomycin/kg bw). Plasma samples were collected at 10 timepoints until 24 hours after treatment. After a 14 day washout period, Group A was dosed by i.m. and Group B by i.v.

In the second study which was a multiple dose experiment, 20 veal calves (60-80 kg), allocated to four groups of five animals each, were given six i.m. injections of 15 mg lincomycin-spectinomycin solution/kg bw. The first two injections were given at 12 hour intervals with the other four injections given every 24 hours. Plasma was collected from one animal from each group with samples collected before the first five injections and at six timepoints up to 12 hours after the sixth injection. The remaining animals were used in a residue depletion study (see summary under Residue Depletion Studies - Cattle).

Rapid absorption of spectinomycin after the single i.m. injection was indicated by the maximum plasma concentration of 20.0 $\pm$ 3.10 μg spectinomycin/ml from 0.33-0.67 hours. The results of the single i.m. and i.v. doses were similar. Mean plasma concentrations of spectinomycin at 12 hours were 0.2 μg/g for i.v. and 0.28 μg/g for i.m. single doses. The AUC after i.v. and i.m. injections were identical, indicating 100% bioavailability of spectinomycin from i.m. injection sites. The terminal phase half-lives for both i.m. and i.v. were approximately 2 hours, indicating rapid elimination from the plasma. Twelve hours after multiple dosing, the mean spectinomycin plasma concentration was 0.4 $\pm$ 0.4 μg/ml which is consistent with the results obtained after single dosing. The pharmacokinetic model was different after multiple dosing compared with the single dose kinetic model, in that there were two depletion rates noted from plasma rather than a single elimination rate constant. The multiple dose model appears to have a more prolonged elimination half-life (Jaglan et al., draft 1993).

Swine

Studies with swine dosed orally and by i.m. with ^{3}H-6'-methyl spectinomycin demonstrated the stability of the label and the distribution of the drug. One male swine (8-10 weeks old, 10 kg bw) was treated i.m. with 10 mg/kg bw of ^{3}H-6'-methyl spectinomycin. A second swine was given a single oral dose of 44 mg/kg bw ^{3}H-6'-methyl spectinomycin. Both animals were kept in metabolism cages. The animals were killed twelve hours after the i.m. treatment and 24 hours after the oral dose. Radioactivity was measured in various tissues. The accountability of ^{3}H-spectinomycin and percent of tritiated water as a percentage of total residues in samples collected from both treatments is shown in Table 2. Small amounts of the dose were expired in the air. Most of the dose from the oral treatment (79%) was still in the GI tract with only about 5% in the urine, indicating that only a small amount of the dose was absorbed as a result of the oral treatment. Most of the dose (55.4%) was excreted in the urine from the intramuscular treatment. More tritiated water was found in tissues from the oral treatment than from the i.m. treatment. Most of the residue in kidneys as a result of the i.m. treatment contained ^{3}H-label in the 6'-methyl position intact and only 1.6% of the residue was tritiated water. However, kidney residues resulting from oral treatment contained 32% tritiated water (Jaglan et al., 1991b; Roof and Jaglan, 1993).

The ^{3}H-spectinomycin had a purity of 87.74% and two major impurities. The identity of one impurity is unknown. The other impurity was spectinomycin that was reduced to hydroxy at the 3'-position in the pyran ring. HPLC analysis of urine from the i.m. dosed swine showed that the drug with its two major impurities was excreted unchanged. HPLC analysis of urine from the orally dosed swine showed tritiated water, spectinomycin, and the two impurities. The unknown impurity may be a metabolite since it was present in the amount of 14.5% compared to 3.5% in the starting material.

Four swine (30-40 kg) were fed a diet medicated with lincomycin-spectinomycin premix at the rate of 44 ppm

each of lincomycin (base) and spectinomycin (base) for seven days. Feces, urine, and blood were collected daily. After seven days treatment, the animals were euthanized and gastrointestinal samples were taken. Samples were analyzed by both microbiological (LOQ 1.06-6.73 μg/g) and HPLC (LOD 1.27-2.0 μg/g) methods (Hamlow et al., 1990). Spectinomycin levels in plasma were measured by GC/MS (LOQ 0.03 μg/ml). Spectinomycin plasma concentrations were below 0.03 μg/ml at all times tested. After seven days of feed medicated with spectinomycin, the largest amount of residues was found in the feces. The microbiological and HPLC methods produced similar results (Davis et al., 1991)

Table 2. **Percent of Dose and Tritiated Water as a Percentage of Total Residues Resulting from Swine dosed orally with 44 mg/kg bw or i.m. with 10 mg/kg bw ^{3}H-Spectinomycin**

	i.m. Treatment		Oral Treatment	
Sample	**% of Dose**	**% Tritiated water**	**% of Dose**	**% Tritiated water**
Volatile	0.05	100	0.32	100
Urine	58.38	0.2	4.46	3.0
Feces	0.04	33.0	0.05	4.3
GI Tract	0.91	17.0	78.62	0.7
Liver	0.86	18.0	0.24	62.0
Kidney	1.11	1.6	0.10	32.0
Muscle	3.69	63.0	2.90	82
Lung	0.21	27.0	0.09	73.0
Plasma	0.63	44.0	0.29	100.0
Total	65.88		87.07	

Table 3. **Mean Residues of Spectinomycin in Swine GI Tract and Plasma (μg/g) following 7-Day Oral Feeding with 44 μg/g Spectinomycin as Lincomycin-Spectinomycin Premix.**

	Microbiological Method	Instrumental Method
Stomach	9.0	7.2
Small Intestine	22.5	14.8
Large Intestine, Ascending	32.2	19.7
Large Intestine, Descending	28.7	33.2
Feces	52.4	49.4
Urine	1.9	1.9
Plasma	-	<0.03

The plasma pharmacokinetics of two groups of swine dosed with 10 mg spectinomycin/kg bw (15 mg/kg bw lincomycin-spectinomycin) were measured by the Hamlow et al. (1990) using a HPLC method (LOQ 0.03 μg/ml) and by the Barbiers et al. (1980) using a microbiological method with a LOD of 2.0 μg/ml. The half life of spectinomycin was calculated to be about 0.98 hours (Davis et al., 1990). When the residue levels measured by HPLC and microbiological methods were compared statistically, no significant difference was found. The authors concluded that this result support that spectinomycin is the main microbiologically active metabolite in plasma for the first 8 hours after treatment (Davis and Hamlow, 1990).

TISSUE RESIDUE DEPLETION STUDIES

Radiolabeled Residue Depletion Studies

The results of the radiolabeled residue depletion studies in swine and cattle with ^{3}H-spectinomycin labeled at the 6'-methyl side chain of the pyran ring showed that kidney has the highest and most persistent residues. Therefore, kidney should be considered the target tissue. Spectinomycin residues in liver were lower than kidney. Muscle and fat had the lowest spectinomycin residues of the tissues analyzed.

Swine

Tissue was obtained from swine treated i.m. and orally as described in the studies by Jaglan et al. (1991b) and Roof and Jaglan (1993) in the Metabolism section. Table 4 shows the concentration of total ^{3}H-spectinomycin tissue residues in the swine resulting from oral and i.m. dosing. The kidney had the highest residues among all tissues in both treatments.

Table 4. Concentration of Total Residues in μg/g ^{3}H-Spectinomycin Equivalents in Swine Tissue as a Result of Dosing with 10 mg/kg bw i.m. or 44 μg/g bw Oral Treatment.

Tissues	12 hours after Intramuscular Injection	24 hours after Oral Treatment
Kidney	21.10	9.36
Liver	3.11	4.99
Lung	1.72	3.95
Muscle	0.82	2.78
Fat	0.78	1.34

Cattle

In the Roof et al. (1993) study summarized under the Metabolism section, total residues were measured in two steers injected i.m. a single time with 22 mg ^{3}H-spectinomycin/kg bw. ^{3}H-spectinomycin equivalents in μg/g found in the tissues of both animals are shown in Table 5. Kidney had the highest residues at both timepoints. The actual residue values are slightly lower since 2.9% of the ^{3}H-spectinomycin was converted to tritiated water (Roof et al., 1993).

Table 5. **^{3}H-Spectinomycin Equivalents (μg/g) in Tissues of Bovine Injected i.m. a Single Time with 22 mg ^{3}H-Spectinomycin/kg bw.**

Time after Treatment	Kidney	Liver	Lung	Muscle
24 hours	40.8	13.3	3.3	1.4
72 hours	40.9	12.3	3.3	0.8

OTHER RESIDUE DEPLETION STUDIES

The results of spectinomycin residue depletion studies indicated that kidney was the target tissue in swine, cattle, and chickens. In swine given daily i.m. injections of 10 mg spectinomycin/kg bw for 7 days, kidney spectinomycin residues were 10.9 μg/g at 12 hours postdose and 0.8 μg/g by 96 hours postdose. In calves given multiple i.m. doses of 10 mg spectinomycin/kg bw, kidney spectinomycin residues were 15.5 μg/g at 8 hours withdrawal and were <0.1 μg/g by 21 days postdose. Spectinomycin levels in milk from dairy cows dosed i.m. with 20 mg/kg bw twice daily for 3 consecutive days were below 0.2 μg/ml at the fifth milking after the last injection.

Swine

Eight swine were give daily i.m. injections of 10 mg spectinomycin/kg bw for 7 days (15 mg lincomycin-spectinomycin). The animals were sacrified at 12, 24, 48, and 96 hours postdose. Kidneys were collected and spectinomycin levels were measured by an HPLC-Thermospray-Mass Spectrometric (LC/MS) method. The limit of detection was 0.02 μg/g. Table 6 shows the spectinomycin residue levels found at four timepoints.

Table 6. **Spectinomycin Residues in Swine Kidney Tissue after Daily i.m. Injections of 10 mg Spectinomycin/kg bw for 7 Days. Residue Values are from one animal.**

Post-Dose in Hours	Spectinomycin Residues in μg/g
12	10.9
12	15.1
24	7.1
24	6.8
48	4.4
48	2.4
96	1.8
96	0.8

This same LC/MS method was used to analyze five swine kidney samples collected from 5 animals 7 days after being orally dosed with 44 μg/g of spectinomycin (88 μg/g lincomycin-spectinomycin premix) in medicated feed. The residue data ranged from <0.02 to 0.07 μg/g (Cazers et al, 1991).

Cattle

Tissue obtained from calves treated in the multiple i.m. dose study described under the Pharmacokinetics section was collected at 0 (8 hours), 7, 14, and 21 days after treatment. Spectinomycin was measured in tissues by the postcolumn derivatization HPLC method (LOD 0.1 μg/g). Table 7 shows the spectinomycin levels in μg/g that were found in each tissue. The highest residues were observed in the kidney at Day 0. Residues at 7 days postdose were undetectable in muscle and fat. Kidney residues declined to <0.1 μg/g at 21 days. At 21 days, only one injection site sample was positive with 0.2 μg/g (Jaglan et al., 1993 draft).

Table 7. Mean Tissue Residues of Spectinomycin (μg/g) in Calves Treated at Various Intervals after Six i.m. Doses with 10 mg Spectinomycin/kg bw.

Withdrawal Time (Days)	Kidney	Fat	Liver	Muscle	Injection Site
0	15.5	0.5	3.3	0.3	5.6
7	1.4	<0.1	0.6	<0.1	0.9
14	0.3	<0.1	0.3	<0.1	0.2
21	<0.1	<0.1	<0.1	<0.1	0.2

Tissue residues in 17 non-ruminating calves injected i.m. six times with 10 mg/kg bw spectinomycin (15 mg lincomycin-spectinomycin/kg bw) were measured with a microbiological method with a limit of detection of 1 μg/g in tissue. Animals were sacrified at 1, 7, 14, 21, and 28 days after treatment. Highest and longest enduring residues were found in kidney. By 14 days postdose, kidney spectinomycin residues were <0.1 μg/g (Barbiers and Smith, 1981).

Milk

Six dairy cows were injected i.m. with 20 mg spectinomycin/kg bw twice daily for 3 consecutive days. Milk samples were collected twice daily, from immediately before to 8 days after the first treatment. Spectinomycin residues were measured by an HPLC method with a 0.2 μg/ml limit of quantitation. Table 8 shows the spectinomycin levels in milk. The mean spectinomycin concentrations in milk were below 0.2 μg/ml by the fifth milking (Day 6, morning milking) after the last injection. No residues were detected in the seventh milking (Day 7, morning milking) after the last injection (Guyonnet, 1991a).

Table 8. Mean Concentrations of Spectinomycin (μg/ml) in Milk following i.m. Treatment with 20 mg Spectinomycin/kg bw Twice Daily for Three Consecutive Days.

Days of milk collection	Spectinomycin in Morning Milking	Spectinomycin in Evening Milking
0	not detected	not detected
1	not sampled	1.26±0.288
2	2.14±0.894	2.82±0.897
3	3.03±0.745	2.62±0.820
4	2.8±0.581	1.67±0.411
5	0.48±0.376	0.34±0.168
6	<0.2*	<0.2*
7	not detected	not detected
8	not detected	not detected

* Milk from two animals had detectable residues.

Milk residues from 12 lactating cows injected i.m. and then i.v. with 20 mg spectinomycin/kg bw/day for 4 days were measured with a microbiological method with a working limit of detection of 0.7 μg/ml. Milk samples were assayed for spectinomycin from 0 to 120 hours after treatment. Spectinomycin residues fell below the limit of detection by 24 hours after each injection (Roberts et al., 1985).

Chicken

Tissue residue in broilers dosed with 2 g spectinomycin/gallon of drinking water were measured by a microbiological method with a limit of detection of 1 μg/g at 6, 12, 18, 24, and 48 hours postdose. Six birds were slaughtered at each timepoint. Kidney samples were taken from pooled tissue of two birds. Highest and longest enduring residues were found in kidney. At 48 hours postdose, 1 out of 3 samples was above the LOD (Barbies et al., 1968b).

Eggs

Residues in eggs from 15 laying hens medicated for 7 days with lincomycin-spectinomycin at either 110, 165, or 220 μg spectinomycin/g feed or with water containing lincomycin-spectinomycin at 500 μg/g were measured by a microbiological method with a detection limit of 2 μg/g. No antimicrobial activity was found in eggs collected on days 8, 9, and 10 post treatment (Keppens and DeSutter, 1992).

METHODS OF ANALYSIS FOR RESIDUES IN TISSUES

Microbiological Assays

Microbiological cylinder plate assays are available to measure spectinomycin levels in plasma, urine, milk, and tissues. The disadvantage of the microbiological assay is that the limit of detection is high, approximately 1 μg/g.

Chromatography Assays

Currently, HPLC is the method of choice for measuring spectinomycin in animal tissue and fluid. An HPLC method has been developed that is able to detect low levels of spectinomycin (limit of detection is about 0.1 μg/g) and is therefore preferable to the microbiological methods.

HPLC method development for the determination of spectinomycin in pharmaceutical formulations began in 1979. It was found that the lack of UV absorbing groups on spectinomycin made it necessary to derivatize the compound prior to spectrophotometrical detection.

Two methods were used as the starting point for the development of procedures to determine spectinomycin in body fluids and tissue. Myers and Rindler (1979) used reverse phase ion-pairing chromatography, followed by post-column oxidation, derivatization with o-phtalaldehyde, and fluorimetric detection. Tsuji and Jenkins (1985) used pre-column derivatization by means of 2-naphthalene sulphonyl chloride and subsequent normal-phase HPLC with UV detection.

Wood et al (1988) developed an assay for spectinomycin in plasma that uses postcolumn derivatization with fluorescence detection. Hamlow et al (1990) modified and validated this method for the determination of spectinomycin in swine plasma. The recovery averaged 97%. The range is linear from 0.01 to 10 μg/g. The CV is 4.4% at 0.01 μg/g and 1% at 10 μg/g. The limit of detection is 0.009 μg/g and the limit of quantitation is 0.030 μg/g.

Cazers et al. (1990) developed an HPLC/Thermospray-MS method for measuring spectinomycin in swine kidney. A loop injection of spectinomycin is used as an external standard for verifying the MS response. The assay employs an acid extraction followed by weak cation exchange column clean-up. The method is linear from 0.05 μg/g to 4 μg/g. The average recovery over that range is approximately 80%. The limit of detection is 0.01 μg/g and the limit of quantitation is 0.10 μg/g.

Haagsma (1991a, b) developed an HPLC assay for spectinomycin in swine, calf, and chicken lissue (muscle, kidney, liver, and fat) with a limit of quantitation of 0.1 μg/g. The method uses postcolumn oxidation with sodium hypochlorite followed by postcolumn derivatization with fluorescence detection and is similar to the procedure described for the method by Myers and Rindler (1979). The calibration curve is linear from 0.5 μg/g to 50 μg/g. The recoveries from fortified calf kidney at 0.1 to 1 μg/g were 79.04±6.61% with a CV of 8.36%. The method also was developed for the determination of spectinomycin in eggs (whole, yolk, white) with a linear response from 0.25 μg/g to 100 μg/g and recoveries of 84.60±3.18% in whole egg fortified at 0.1 to 10 μg/g.

Guyonnet (1991b) developed an HPLC for the determination of spectinomycin in tissues, eggs, and milk. The method uses precolumn derivatization with 9-fluorenylmethyl chloroformate and fluorescence detection. The method was validated for swine, poultry and cattle tissues, eggs, and cow milk. The limit of quantitation was 0.2 μg/g or μg/ml and the limit of detection was 0.1 μg/g in liver, kidneys, muscle, fat, eggs, and milk. The recovery rate at the limit of quantitation was 102% for calf kidneys, 72% for swine kidneys, 79.5% for chicken muscle, 42% for eggs, and 72.5% for cow milk.

APPRAISAL

Spectinomycin is poorly absorbed after oral administration, with a bioavailability factor of about 10%. In contrast, spectinomycin is well absorbed after intramuscular injections. The absorbed fraction has a low volume of distribution and is mainly eliminated in the urine in the form of unchanged and biologically active spectinomycin. The renal elimination and the poor (if any) metabolism of spectinomycin are related to its high hydrosolubility. Elimination from plasma and tissues is therefore relatively rapid and repeated administrations fail to induce drug accumulation. For the same reasons, the therepeutic scheme for systemic disease must include frequent injections.

A relatively rapid elimination rate was shown in edible tissues, whatever the animal species. Residue depletion studies performed at therapeutic dose levels in swine, poultry and cattle show that residues of the marker residue spectinomycin are present in the edible tissues at concentrations exceeding the proposed MRLs for periods not exceeding 10 days. The highest levels are achieved in kidneys and, at a lower degree, in liver. Lower concentrations are obtained in fat and muscle. Spectinomycin fails to reach high concentrations in milk, leading to short withdrawal periods (about 4 milkings). The tissue elimination half-times of spectinomycin range from 0.7 to 6 days, the lowest values being observed in poultry kidneys. As the highest concentrations are measured in kidneys, whatever the animal species, this organ should be considered as the target tissue for residue monotoring in carcasses.

Methods of analysis at the proposed MRLs for spectinomycin are available. Microbiological assays are capable of measuring the marker residue (spectinomycin) in the target tissue (kidney) at the recommended MRL. A variety of chromatographic assays are available to measure spectinomycin in all tissues and milk.

As much of the residue depletion data reviewed by the Committee were either interim progress reports or pilot studies, the Committee recommends only temporary MRLs to be given to spectinomycin until the final reports are received. Additionally, the Committee is aware that additional metabolic studies are being done to confirm that the microbiological portion of the residue in edible tissues is predominantly spectinomycin. These studies are of particular importance considering the paucity of tissue depletion data in poultry.

Maximum Residue Limits

Based on the ADI of 0-40 μg/kg body weight for the parent drug established by the Committee, the permitted daily intake of parent drug is 2400 μg.

The Committee recommends the following temporary MRLs for swine, cattle and chickens: kidney 5000 μg/kg, liver 2000 μg/kg, muscle 300 μg/kg, and fat 500 μg/kg. The temporary MRL recommended for milk is 200 μg/l.

Using these values for MRLs, and daily consumption of 300 g of muscle, 100 g of liver, 50 g of kidney, 50 g of fat and 1.5 l of milk, the theoretical maximum daily intake of spectinomycin residues is 865 μg/day.

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