PIRLIMYCIN

First draft prepared by

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IDENTITY

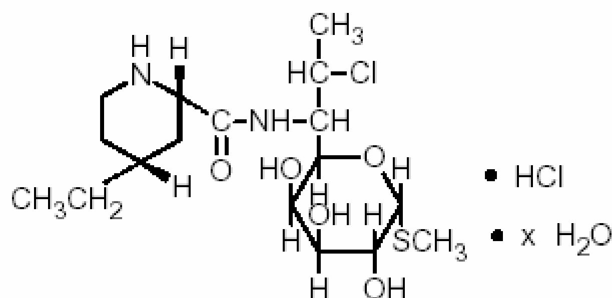
Chemical Names: (2S-*cis*)-Methyl 7-chloro-6,7,8-trideoxy-6-[[(4-ethyl-2-piperidinyl)carbonyl]amino]-1-thio-L-*threo*-alpha-D-galactooctopyranoside monohydrochloride, hydrate

Synonyms: Pirlimycin hydrochloride

PIRSUE® Sterile Solution

PNU-57930E

Structural formula:



Molecular formula: $C_{17}H_{31}O_5N_2ClS$ • HCl • xH_2O

Molecular weight: 447.42 (without the water of hydration)

OTHER INFORMATION ON IDENTITY AND PROPERTIES

Pure active ingredient: Pirlimycin

Appearance: White crystalline powder

Melting point: 210.5 – 212.5°C with decomposition

Solubility (g/L) of Pirlimycin:	pH dependent aqueous:	70 at pH 4.5
		3 at pH 13
	Protic organic solvents:	≥ 100
	Other organic solvents:	≤ 10

Optical rotation: +170° to +190°

UV$_{max}$: >220 nm

RESIDUES IN FOOD AND THEIR EVALUATION

Conditions of use

General

Pirlimycin hydrochloride is a lincosamide antibiotic with activity against the Gram-positive organisms. Pirlimycin has been shown to be efficacious for the treatment of mastitis in lactating dairy cattle caused by sensitive organisms such as *Staphylococcus aureus, Streptococcus agalactiae, S. uberis* and *S. dysgalactiae*. The general mechanism of action of the lincosamides (lincomycin, clindamycin and pirlimycin) is inhibition of protein synthesis in the bacterial cell, specifically by binding to the 50s ribosomal subunit and inhibiting the peptidyl transferase, with subsequent interference with protein synthesis.

Dosage

The optimum dose rate for pirlimycin has been established as 50 mg of free base equivalents per quarter administered twice at a 24-hour interval by intramammary infusion of a sterile aqueous solution formulation. For extended therapy, daily treatment may be repeated for up to 8 consecutive days.

PHARMACOKINETICS AND METABOLISM

Pharmacokinetics in Laboratory Animals

Rats

Rats were treated with an oral gavage dose of 30 mg of ^{14}C-pirlimycin per kg of body weight as an aqueous formulation at 24-hour intervals for 5 consecutive days (Nappier, 1989). All animals were sacrificed at 2 to 4 hours after the last treatment. Approximately 88% of the administered dose was recovered in urine, feces and gastro-intestinal tract contents as shown in Table 1. There were no significant differences between male and female rats.

Table 1 Excretion of total ^{14}C-pirlimycin after dosing rats with 30 mg/kg/day for 5 days

	Percent of Total ^{14}C-Pirlimycin Dose	
Sample	Male rats	Female rats
Urine	4.5	6.4
Feces	62.8	58.8
Gastrointestinal Tract	20.6	22.5
Total	87.9	87.7

Mice

Pharmacokinetic studies were not conducted in mice.

Pharmacokinetics in Food Animals

General

The three studies have been conducted to examine the absorption, distribution, metabolism, and excretion of pirlimycin in the dairy cow following intramammary infusion of ^{14}C-pirlimycin (Hornish, 1988; Hornish, 1989a; Hornish, 1989b; Hornish, 1992a; Hornish, 1993c; Hornish, 1993d). Pirlimycin was readily labelled in the carboxyl carbon of the amide linkage and had a specific activity of 11.7 mCi/mmole (433 MBq/mmole) and a radiochemical purity of >98% (Hornish, 1988). The selection of this label site was based on the known metabolism of lincomycin and clindamycin (lincosamides structurally related to pirlimycin) in the dog, man, and rat (Daniels, 1976; Daniels, 1977; Eberts, 1967; Hornish, 1987; Onderdonk, 1981; Sun, 1973a; Sun, 1973b). These studies indicate that the lincosamides are not metabolized by cleavage of the amide linkage, which would expose the carbonyl carbon to subsequent metabolism and potential loss as carbon dioxide. Studies conducted in dairy cattle have demonstrated that this labelling site is metabolically stable for the complete delineation of the metabolism and residue fate of pirlimycin in the cow (Hornish, 1988).

Cattle

A GLP study was conducted in which 12 dairy cattle in mid-lactation were treated with ^{14}C-pirlimycin hydrochloride by intramammary infusion twice at a 24-hour interval at a dose of 200 mg/quarter (Hornish, 1988; Hornish, 1989a; Hornish, 1989b). This is four times the recommended dose. Blood samples were taken by jugular venipuncture at the times indicated in Table 2 and the total ^{14}C-pirlimycin free-base equivalents determined by combustion analysis of the whole blood (Hornish, 1988).

Table 2. **Concentration of total ^{14}C-pirlimycin residues as a function of time in whole blood of dairy cows administered ^{14}C-pirlimycin by intramammary route at a dose of 200 mg/quarter twice at a 24-hour interval in each quarter**

Sample Time Dose + Hour	Number of Data Points per Sample Time	Mean Concentration in Blood (µg/L)
D1 + 0.5	6	6
D1 + 1.0	12	11
D1 + 2.0	12	19
D1 + 4.0	12	38
D1 + 6.0	12	55
D1 + 8.0	6	53
D1 + 9.0	6	86
D1 + 10.0	6	64
D1 + 12.0	12	83
D1 + 16.0	12	54
D1 + 24.0	12	37
D2 + 6.0	12	119
D2 + 12.0	12	126
D2 + 24.0	12	63
D2 + 36.0	8	44
D2 + 48.0	12	38
D2 + 72.0	3	38

Concentrations in the blood were low but indicated that some of the drug was absorbed into the systemic circulation. The elimination phase suggested a bi-phasic pharmacokinetic model. Blood residues were not metabolically profiled. Analysis of the milk and urine samples collected during the terminal depletion phase showed that these samples contained >95% and >80% parent pirlimycin, respectively, suggesting that the blood residue was most likely composed of parent pirlimycin. Mean pharmacokinetic parameters were estimated following non-compartmental analysis (Hornish, 1988). Results are presented in Table 3.

Table 3 **Whole blood pharmacokinetics of ^{14}C-pirlimycin total residue in the dairy cow following intramammary administration of 200 mg/quarter**

Parameter	Value
AUC_{0-120}	2.27 to 7.11 µg-hr/mL
$t_{½}$ of abs. phase	2.89 ± 0.46 hours
C_{max-1}	0.083 ± 0.030 ppm
C_{max-2}	0.131 ± 0.047 ppm
K_{el}	0.0224 ± 0.009 hr^{-1}
$t_{½}$ of terminal phase	37.6 ± 17.4 hrs

The animals in the study were sacrificed at 4, 6, 14, and 28 days after last treatment (Hornish, 1988). Total milk at 12-hour intervals and urine and feces at 24-hour intervals were collected through 6 days after last treatment or until the animal was sacrificed. Total liver, kidney, udder, and samples of abdominal fat and flank and udder diaphragm muscle were harvested for total residue and metabolite determination. The results are presented in Table 4.

Table 4 Disposition and accountability of ^{14}C-pirlimycin total residue in the dairy cow following intramammary administration of 200 mg/quarter

Withdrawal Time (days)	Mean Percent of Total Administered Dose				
	Milk	Urine	Feces	Tissues[1]	Total
4	51.6	7.6	22.8	8.9	90.9
6	58.7	10.4	18.3	5.8	91.2
14	42.3	9.4	30.2	2.4	84.3
28	50.9	12.2	23.8	0.3	87.2
MEAN	50.9	9.9	23.8	4.4[2]	88.9

[1] Calculated from weight of whole liver, kidneys, udder and estimated muscle and fat weights as 55% and 25%, respectively, of total body weight at slaughter.

[2] Mean residue concentration over the withdrawal time range in tissues is for computation only and has no physiological significance.

Approximately 50% of the total dose was transported to the systemic circulation. Nearly 10% of the total dose was excreted via the urinary tract and 24% of the total dose was excreted via the GI tract through the 4 to 6 days of collection.

The depletion of total residue from the milk in the dairy cow studies was bi-phasic. A rapid initial phase was caused by unabsorbed pirlimycin being flushed from the udder during the first 3 or 4 milkings post-treatment (Hornish, 1988; Hornish, 1992a).

In a second GLP study (Hornish, 1992a; Hornish, 1993c), 23 cows were treated twice at a 24-hour interval in all four quarters with 50 mg ^{14}C-pirlimycin /quarter. The disposition of the total administered dose in milk (50.7%), urine (12.7%), feces (27.6%) and tissues (4.6%) gave an overall accountability of 95.7%.

In a third non-GLP study, three healthy lactating dairy cows (Hornish, 1993d) in mid-lactation were treated intravenously with a single infusion of 811 mg of ^{14}C-pirlimycin hydrochloride in sterile water. Blood samples were collected over a 7-day period. Following a four-week washout period, the cows received an intramammary infusion of 790 - 795 mg of ^{14}C-pirlimycin, approximately 200 mg in each quarter. Blood samples were again collected through 7 days. In addition, all milk, urine, and feces were collected for 7 days post-treatment following each dose. All samples were assayed for total radioactivity and for parent pirlimycin. The total residue results are summarized in Tables 5 and 6 (intravenous and intramammary administration, respectively) and the parent pirlimycin residue results are summarized in Table 7.

Table 5 Pharmacokinetics and disposition of total pirlimycin after intravenous (IV) administration of ^{14}C-pirlimycin to lactating dairy cows

Parameter	Cow 589	Cow 590	Cow 592
Model/Best Fit	Triexponential	Triexponential	Triexponential
A (ng/mL)	778.5±31.9	1547.5±145.4	794.1±124
Alpha (hr^{-1})	1.59±0.17	2.29±0.54	3.04±0.71
B (ng/mL)	293.2±46.7	270.9±200.6	342.0±132
Beta (hr^{-1})	0.06±0.02	0.09±0.12	0.54±0.24
C (ng/mL)	23.2±53.2	37.5±224.2	173.1±21.0
Gamma (hr^{-1})	0.004±0.019	0.01±0.07	0.018±0.004
$T_{½}\alpha$ (hours)	0.44	0.30	0.23
$T_{½}\beta$ (hours)	11.6	8.1	1.3
$T_{½}\gamma$ (hours)	173.3	70.0	38.5
$AUC_{0-\infty}$ ng*min/mL	10911.9	7642.5	10615.1

Table 6 Pharmacokinetics and disposition of total pirlimycin after intramammary (IMM) administration of ^{14}C-pirlimycin to lactating dairy cows

Parameter	Cow 589	Cow 590	Cow 592
Model/Best Fit	Triexponential[1]	Triexponential[1]	Biexponential[2]
A (ng/mL)	1771.8±63547	172.8±1440	
Ka (hr^{-1})	0.16±0.47	0.08±0.23	
B (ng/mL)	-1847.7±63551	-179.6±1457.5	110.3±66.2
Alpha (hr^{-1})	0.19±0.51	0.14±0.35	0.15±0.11
C (ng/mL)	49.0±20.8	27.4±31.7	623.5±231
Beta (hr^{-1})	0.01±0.005	0.005±0.009	0.01±0.006
$T_{½}Ka$ (hours)	4.2	8.7	
$T_{½}\alpha$ (hours)	3.6	4.6	4.9
$T_{½}\beta$ (hours)	58.1	69.3	60.2
$AUC_{0-\infty}$ ng*min/mL	5157.2	6411.4	6072.9

[1] with 1st-order absorption
[2] with 0-order absorption

Table 7 Pharmacokinetics and disposition of parent pirlimycin after intravenous (IV) and intramammary (IMM) administration of ^{14}C-pirlimycin to lactating dairy cows

Parameter	IV	IMM
Dose (mg)	811	790-795
C_{max} (ng/mL)	N/A	62-96
T_{max} (hours)	N/A	9
$AUC_{0-\infty}$ (ng/mL/min)	3528-5510	1435-1868
Cl_B (mL/hr)	$1.47\text{-}2.3 \times 10^5$	N/A
$T_{½}\alpha$ (hours)	0.16-0.27	10.5-12.6
$T_{½}\beta$ (hours)	10.8-23.1	
T_{abs} (0-order in hours)		7.2-7.9
MRT (hours)	17.9-33.7	
Vss (L)	4110-4960	
Excretion Recovery:	**IV**	**IMM**
% in milk	4.3 ± 0.7	40.2 ± 16.6
% in urine	26.5 ± 3.0	12.5 ± 2.6
% in feces	47.1 ± 1.7	29.7 ± 8.9
Total recovery	77.8 ± 2.2	82.5 ± 8.4

The bioavailability of pirlimycin in cattle following intramammary infusion was calculated to be 34% to 41%. The percent absorbed, measured as total ^{14}C-pirlimycin, residues was 51%.

Metabolism in Toxicological Test Species

Rats

The metabolism of pirlimycin was evaluated in the rat, the primary species used in the toxicological testing. Rats were treated by oral gavage once daily for 5 days with a dose of 30 mg of ^{14}C-pirlimycin per kg of body weight (Nappier, 1989) and sacrificed at 2 to 4 hours after the last treatment.

Liver was the tissue with the highest total residues and parent pirlimycin and the sulfoxide metabolite were the only residues found.

Mice

Metabolism studies were not conducted in mice.

Metabolism in Food Animals

Cattle

In the GLP study in which cows were treated by intramammary infusion twice at a 24-hour interval at a dose of 200 mg/quarter, milk samples were collected and analyzed by both an HPLC method and by a microbiological method (Hornish, 1989a). The results indicate that unchanged pirlimycin (by HPLC) comprised nearly 95% of the total residue in the milk, but the microbiologically active component in the milk was 106% of parent pirlimycin concentration measured by HPLC. Nearly all of the nonpirlimycin was found in the Dose + 12 hour samples and was attributed partially to unknown spurious spikes and partially to pirlimycin sulfoxide. These components contributed negligible amounts to total residue in other samples.

Residues in cattle liver were also examined by the two methods mentioned above (Hornish, 1989a). The HPLC analysis indicated that the residue consisted of only two components: pirlimycin sulfoxide as the major residue (76.5%) and unchanged pirlimycin as the minor residue (21.9%). The data demonstrate that the relative amounts varied over time, but are fairly constant in the critical 4-6 day withdrawal period as shown in Table 8. Parent pirlimycin is an acceptable residue marker since it is the only microbiologically active residue and is readily analyzed by a variety of methods.

Table 8 Percentage of total pirlimycin residue that is microbiologically active or parent pirlimycin in dairy cow liver following two intramammary infusions of 200 mg/quarter of ^{14}C-pirlimycin

Withdrawal Time (days)	Mean Percent of Pirlimycin in Liver by	
	M. luteus	HPLC/RAM
4	22.3	24.3
6	25.7	34.0
14	9.0	13.3
28	16.0	38.7

The metabolic profile of pirlimycin in the dairy cow for milk, liver, urine and feces (Hornish, 1989b) is summarized in Table 9.

The metabolism of pirlimycin was relatively simple. Pirlimycin sulfoxide was the only major metabolite isolated and was likely produced by oxidative hepatic processes. The sulfoxide, although the major residue in liver (65-75%), comprised only 5% of the excreted residue. The other pirlimycin residues identified in dairy cow liver were parent pirlimycin (22-25%) and pirlimycin sulfone (9.5%). Residues in the urine are about 80% pirlimycin and 8% sulfoxide; residues in the feces are about 45% parent and 2% sulfoxide. The remainder in urine and feces consists of adenylated adducts of pirlimycin and pirlimycin sulfoxide (Hornish, 1989b). Pirlimycin sulfoxide has approximately 1/100 (or 1%) of the microbiological activity of pirlimycin itself (Kennedy, 1991; Yancey, 1990; Yein, 1989a).

Table 9 Metabolic profile of the pirlimycin residues in the dairy cow following two intramammary infusions of 200 mg/quarter of ^{14}C-pirlimycin

Sample	Mean Percent Composition of Total Residue[1]		
	Pirlimycin	Sulfoxide	Other[2]
Milk	>95	<5	
Liver[3]	≈22	≈77	
Urine[4]	≈80	≈8	≈11
Feces[4]	≈45	≈2	≈50

[1] Metabolite composition in each sample, not percent of total dose
[2] Comprised of adenylated adducts of pirlimycin and pirlimycin sulfoxide
[3] Average of 11 cows at 4, 6, 14, and 28 days withdrawal
[4] Average of 12 cows through 4-6 days post last treatment.

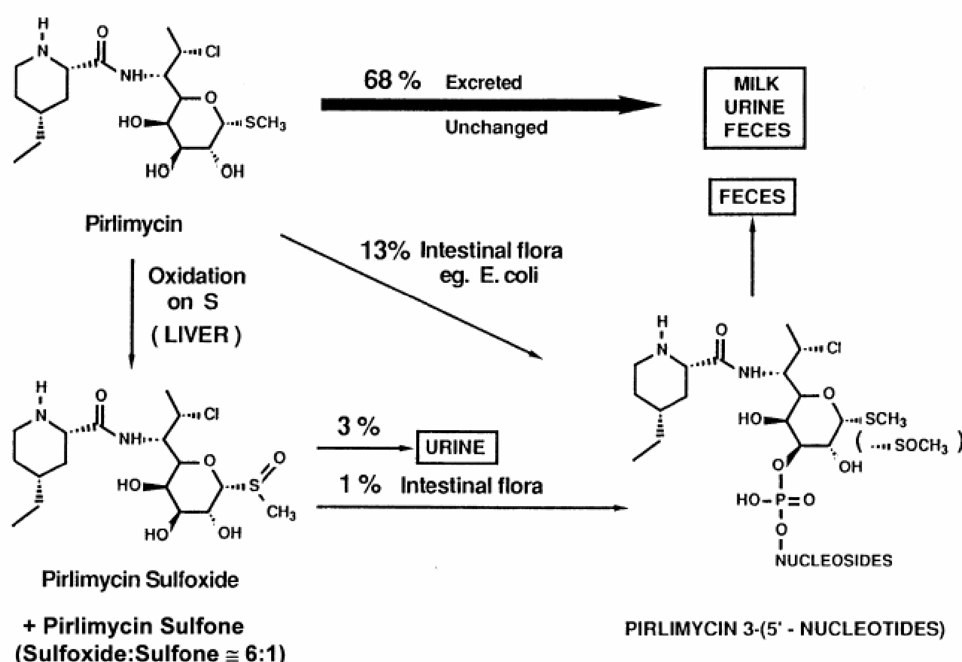
In cows dosed twice at a 24-hour interval in all four quarters with 50 mg ^{14}C-pirlimycin/quarter (Hornish, 1992a; Hornish, 1993c), four components were found in the liver metabolite profile. These were identified as pirlimycin (24.5%), pirlimycin sulfoxide (61.8%), and pirlimycin sulfone (9.8%).

Metabolites were identified in kidneys from four cows (three cows slaughtered 6 days after last treatment and one cow slaughtered 14 days after last treatment) (Hornish, 1993c). The mean composition of metabolites was 43.0% parent pirlimycin, 46.1% pirlimycin sulfoxide, and 7.2% pirlimycin sulfone. This composition is qualitatively similar to the composition in the liver. The concentration of total residue in the kidney was less than one-tenth the concentration in the liver at 10 days or more after last treatment.

Based on the studies described above, the metabolism of pirlimycin in the dairy cow resulting from the infusion of an aqueous solution of pirlimycin into the udder (intramammary route) is summarized in Figure 1 (Hornish, 1992b).

The various metabolites and residues of pirlimycin collected in milk, tissues, urine and feces all have significantly less microbiological activity (< 1%) than parent pirlimycin itself (Kennedy, 1991; Yancey, 1990; Yein, 1989a). Thus, parent pirlimycin is the key residue from a microbiological perspective and is an appropriate target analyte for residue monitoring purposes.

Figure 1 The metabolism scheme for pirlimycin in the dairy cow following intramammary administration of pirlimycin hydrochloride.



Based on the studies described above, liver is the tissue with the highest total residues of pirlimycin in rats and cattle. Parent pirlimycin and the sulfoxide were the only residues found, though the ratio of pirlimycin to pirlimycin sulfoxide was higher in the rat than in the cow. There was a good qualitative match of urine metabolites as well, but the two minor metabolites found in cow urine were not seen in the rat urine. Significant differences were observed in the fecal metabolite profiles, but those metabolites found in the cow feces that were not found in the rat feces have been postulated to arise from gut microflora deactivation and not from animal metabolism. These metabolites are not available to human consumers. The rat is considered a suitable species for toxicity testing of pirlimycin and its metabolites.

TISSUE RESIDUE DEPLETION STUDIES

Radiolabelled Residue Depletion Studies

Cattle

A GLP-compliant tissue residue depletion study (Hornish, 1992a; Hornish, 1993c) was conducted to determine the concentration of pirlimycin and total pirlimycin-related residues in the tissues of lactating dairy cows after treatment twice at a 24-hour interval with ^{14}C-pirlimycin in all four quarters at 50 mg/quarter. This is the recommended dose. A total of 23 cows were used in the study. Cows were slaughtered and tissues were harvested at 6, 10, 14, and 18 days after the last dose.

The disposition of the total administered dose in milk (50.7%), urine (12.7%), feces (27.6%) and tissues (4.6%) gave an overall accountability of 95.7%, as described above. The concentrations of total ^{14}C-residue found in the various tissues at the four slaughter time points are provided in Table 10.

Table 10 Mean residues of ^{14}C-pirlimycin in tissues of cows treated with 50 mg pirlimycin /quarter into all 4 quarters twice at a 24-hour interval

Post-treatment Interval, days (# cows)	Mean Concentration of Total ^{14}C-Pirlimycin Residue (µg/kg)*			
	Liver	Kidney	Muscle	Fat
6 d (n = 5)	2180 ± 1210	300 ± 210	18 ± 11	10 ± 10
10 d (n = 5)	1890 ± 1230	150 ± 80	11 ± 4	10 ± 10
14 d (n = 8)	990 ± 55	60 ± 40	7 ± 7	6 ± 2
18 d (n = 5)	890 ± 72	40 ± 30	< 5	< 5

*By combustion analysis and liquid scintillation counting.

Liver contains the highest residue at all time points. The concentration of total residue in the kidney was less than one-tenth the concentrations in the liver at 10 days or more after last treatment. Muscle and fat contain negligible concentrations of residue.

Residue Depletion in Milk

The same 23-cow GLP-compliant radiolabelled residue study was used to evaluate residues of pirlimycin in milk. The various milk samples collected throughout the study were analyzed for total ^{14}C-residues by scintillation counting procedures and for pirlimycin itself by the microbiological cylinder-plate analysis method (Yein, 1989b). The results of these analyses, Table 11, indicated that unchanged pirlimycin comprised >92% of the total residue "excreted" in milk by the microbiological cylinder-plate assay used in this study.

Table 11 Mean residues of ^{14}C-pirlimycin and Ratio of Parent Pirlimycin to Total Pirlimycin Residue in milk of cows treated with 50 mg pirlimycin /quarter into all 4 quarters twice at a 24-hour interval

Time (Hours) Post-treatment	Mean Pirlimycin Concentration, µg/kg		Ratio ‡
	Total residue *	*M. luteus* †	
Dose 1 + 12	19500	18000	0.91
Dose 1 + 24	2670	2470	0.90
Dose 2 + 12	18400	17000	0.93
Dose 2 + 24	2030	1770	0.89
Dose 2 + 36	420	380	0.90
Dose 2 + 48	170	150	0.93
Dose 2 + 60	110	100	0.96
Dose 2 + 72	80	70	0.95

* Concentration of total ^{14}C-residue determined by Liquid Scintillation Counting.

† Concentration of the microbiological activity (pirlimycin equivalents) based on the microbiological assay, not corrected for 95% recovery factor of the method.

‡ Based on the ratio of 23 samples per time point, not the ratio of the means.

Residue Depletion studies with unlabelled drug

Cattle - tissue residues

Three GLP-compliant studies were conducted to evaluate depletion of unlabelled pirlimycin in the tissues of cows.

In the first study, healthy cows were treated in either 2 (24 cows) or 4 (33 cows) quarters twice in a 24-hour period at a dose of 50 mg pirlimycin/quarter (Hornish, 1993a). The cows were slaughered at each of four time points (7 [4 quarter-treated only], 14, 21 and 30 days) after the last treatment. Liver residues were determined using the HPLC/TSP/MS without incubation and with the cylinder plate microbiological assay. The results are summarized in Table 12.

Table 12 Mean pirlimycin concentration (µg/kg) in cattle liver at each time point after 2 treatments with 50 mg pirlimycin in either 2 or 4 quarters

Withdrawal (days)	Treatment		
	2 quarters	4 quarters	Assay
7	- -	490±150 (430±110)	HPLC/TSP/MS (Cylinder plate)
14	90±40 (50±30)	70±30 (80±70)	HPLC/TSP/MS (Cylinder plate)
21	40±10 (30±10)	40±10 (60±30)	HPLC/TSP/MS (Cylinder plate)
30	50±10 (30±10)	60±30 (40±20)	HPLC/TSP/MS (Cylinder plate)

In the second study, four healthy cows were slaughtered at each of four time points (2, 7, 14, 21, and 28 days) after two treatments in all four quarters with 50 mg of pirlimycin (Hornish, 1997b). The results are summarized in Table 13. The table includes the results for “incubated” liver. This incubation step, which treats a subsample of liver at 37°C for 24 hours prior to the extraction step, was added to the sample preparation process when it was shown that the liver metabolite composition could change during sample preparation resulting in a reversion of pirlimycin sulfoxide to parent pirlimycin. This reversion was likely driven by residual enzyme activity left in the liver after necropsy (Hornish, 1998a; Hornish, 1998b; Hornish, 1998e).

Table 13. Mean pirlimycin concentration (µg/kg) * in 4 cows at each time point after 2 treatments with 50 mg pirlimycin in each quarter

Withdrawal (days)	Liver		Kidney	Muscle	Fat	Udder
	No Incubation	Incubation (24 hr at 37°C)				
2	1470±220	1690±210	460±70	20±30	<LOQ†	1040±350
7	240±40	610±190	60±10	<LOQ	<LOQ	150±120
14	<LOQ†	210±120	<LOQ	<LOQ	<LOQ	<LOQ
21	<LOQ	60±60	<LOQ	<LOQ	<LOQ	<LOQ
28	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ

* HPLC/TSP/MS method
† LOQ = 25 µg/kg

In the third study, cows were treated for eight days (Hornish, 2000). In this study 5 cows were slaughtered at each post treatment time period. The results are presented in Table 14. Again, the table includes the results for "incubated" liver.

Table 14 Mean pirlimycin concentration (µg/kg)* in 5 cows at each time point after 8 treatments with 50 mg pirlimycin in each quarter

Withdrawal (days)	Liver		Kidney	Muscle	Fat	Udder
	No Incubation	Incubation (24 hr at 37°C)				
21	32±21	165±81	<LOQ†	<LOQ	<LOQ	<LOQ
28	21±22	165±182	<LOQ	<LOQ	<LOQ	<LOQ
35	28±18	96±67	NA‡	NA	NA	NA
42	<LOQ	42±46	NA	NA	NA	NA

* HPLC/TSP/MS method
† LOQ = 25 µg/kg
‡ Not assayed

In addition to the residue depletion studies conducted in healthy, non-mastic cows, an additional study was conducted in cows with an induced mastitis. These cows were then treated with four different regimens (Hornish, 1998d). Although these treatments were intended to evaluate the effectiveness of various extended-therapy regimens, animals were slaughtered and liver residue data were evaluated to assess whether the presence of mastitis affected the residue concentrations in the liver. All of the cows received a dose of 50 mg pirlimycin/quarter into all 4 quarters from one of the following treatment regimes: 2 doses at a 24-hour interval (8 cows); 5 doses at a 24--hour interval (8 cows); 8 doses at a 24-hour interval (8 cows); 6 doses with 36 hours between two consecutive daily doses at a 24-hour interval (8 cows). Samples of liver were assayed for pirlimycin residue using the HPLC/TSP/MS assay. These data are summarized in Table 15.

Table 15 Mean Pirlimycin Concentration (µg/kg)* in the Livers of Mastitic Cows at Each Time Point After various 50 mg Pirlimycin treatments in Each Quarter

Time after last treatment (days)	2 Doses	5 Doses	8 Doses	6 Doses
8		1000±230 (n=4)		1880 (n=1)
10	370 (n=1)			
15				750 (n=1)
16			50 (n=1)	
29	70±90 (n=7)	70±20 (n=4)	80±80 (n=7)	90±50 (n=6)

* HPLC/TSP/MS method (LOQ = 25 µg/kg)

The data for this study are insufficient to compute decline curves but suggest that residue depletion is similar in healthy and mastitic cows. Additionally, the data suggest that extended therapy does not greatly increase residues in the liver after several days of withdrawal.

Milk Residues

Three GLP-compliant studies were conducted to evaluate depletion of unlabelled pirlimycin in the milk of cows. In the first study, cows were treated in two quarters with pirlimycin at a dose of 50 mg/quarter twice in a 24-hour period (Hornish, 1993a). Milk residues were determined using the cylinder plate microbiological assay. Additionally, tissues were assayed using several screening tests. The results are summarized in Table 16.

Table 16 Mean residues (µg/kg) of pirlimycin in milk of cows following two daily intramammary doses of pirlimycin HCl at 50 mg/quarter into 2 quarters

Sample Time	Screening Test			
	Cylinder Plate (20 µg/kg)*	BSDA (70 µg/kg)	Delvotest-P (100 µg/kg)*	Charm II Macrolide (30 µg/kg)*
D2+12	4720±3050	32/32	32/32	32/32
D2+24	380±260	23/32	32/32	32/32
D2+36	100±50	7/32	6/32	32/32
D2+48	50±20	0/32	0/32	22/32
D2+60	30±10	0/32	0/32	12/32
D2+72	20±10	0/32	0/32	5/32

* estimated LOD of method

In the two- and eight-dose studies (Hornish, 1997a; Hornish, 2000) milk residue concentrations also were determined. Data from the two-dose study are summarized in Table 17 and residues from the eight-dose study are summarized in Table 18.

The concentrations determined using the cylinder plate assay and the HPLC/TSP/MS assay were nearly the same throughout the study, indicating that the pirlimycin residue measured using the HPLC/TSP/MS method corresponds to the microbiological residue measured with the bioassay.

Table 17 Mean residues of pirlimycin in milk of cows (n=20) following two daily intramammary doses of pirlimycin HCl at 50 mg/quarter into all 4 quarters

Sample Time	Pirlimycin concentration (µg/kg)	
	Cylinder Plate Assay*	HPLC/TSP/MS Assay**
Dose 1 + 12 hr	10300±4430	10300±4650
Dose 1 + 24 hr	820±1200	770±880
Dose 2 + 12 hr	13600±7180	10400±4990
Dose 2 + 24 hr	770±860	820±760
Dose 2 + 36 hr	220±230	210±310
Dose 2 + 48 hr	100±60	110±70
Dose 2 + 60 hr	50±20	70±20
Dose 2 + 72 hr	30±20	50±20
Dose 2 + 84 hr	30±10	(30±10)†
Dose 2 + 96 hr	20±10	(20±10)†

* LOQ = 20 µg/kg; LOD = 20 µg/kg ** LOQ = 50µg/kg; LOD = 20 µg/kg

† Values less than LOQ but greater than LOD

Table 18 Mean residues of pirlimycin in milk of cows (n=20) following eight daily intramammary doses of pirlimycin HCl at 50 mg/quarter into all 4 quarters

Sample Time	Pirlimycin concentration (µg/kg)*
Dose 8 + 12 hr	18600±12200
Dose 8 + 24 hr	1890±1800
Dose 8 + 36 hr	450±330
Dose 8 + 48 hr	160±40
Dose 8 + 60 hr	120±50
Dose 8 + 72 hr	80±30
Dose 8 + 84 hr	80±30
Dose 8 + 96 hr	50±20
Dose 8 + 108 hr	40±20

* Cylinder Plate Assay: LOQ = 20 µg/kg; LOD = 20 µg/kg

When the 2-dose and 8-dose treatments were compared, the depletion profiles for milk residues were not substantially different. The 2- and 8-dose treatments are compared in Table 19.

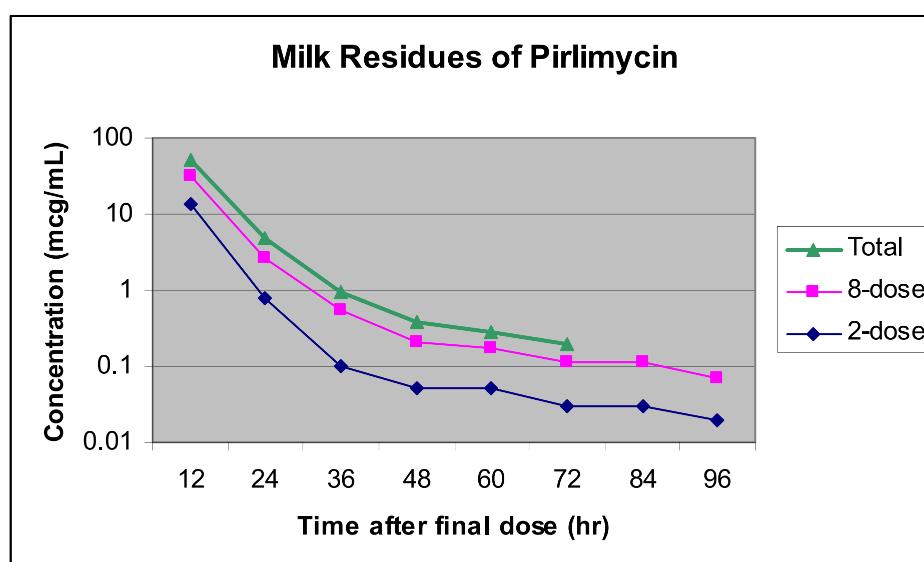
Table 19 Comparison of mean pirlimycin residues in the milk of cows following intramammary treatment at 50 mg/quarter into all 4 quarters for either 2 days or 8 days

Milk Sample	Pirlimycin concentration (µg/kg)*	
	2-Doses	8-Doses
12 hr after last treatment	13600±7180	18600±12200
24 hr after last treatment	770±860	1890±1800
36 hr after last treatment	220±230	450±330
48 hr after last treatment	100±60	160±40
60 hr after last treatment	50±20	120±50
72 hr after last treatment	30±20	80±30
84 hr after last treatment	30±10	80±30
96 hr after last treatment	20±10	50±20

* Cylinder Plate Assay: LOQ = 20 µg/kg; LOD = 20 µg/kg

Residues resulting from the 8-dose treatment were consistently higher (approximately 2X) than the residues resulting from the 2-dose treatment. This is shown graphically in Figure 2.

Figure 2: Mean concentrations of pirlimycin, determined using the cylinder plate bioassay, following 2-dose or 8-dose treatments at 50 mg pirlimycin/quarter into all 4 quarters and the total ^{14}C-pirlimycin residues from the radiolabelled depletion study.



Residues from the extended therapy study in mastitic cows are summarized in Table 20.

Table 20 Mean residues of pirlimycin in milk of mastitic cows (n=8) following various intramammary treatment regimes with pirlimycin HCl at 50 mg/quarter into all 4 quarters

Milk Sample	Pirlimycin concentration (µg/kg)			
	2 Doses	5 Doses	8 Doses	6 Doses
12 hr after last dose	6610±2340	7740±2080	6300±1710	5840±670
24 hr after last dose	420±90	990±420	650±340	450±100
36 hr after last dose	200±30	290±110	260±80	220±60
48 hr after last dose	100±20	120±40	120±30	100±30
60 hr after last dose	80±20	90±30	90±30	80±20
72 hr after last dose	60±20	70±10	70±30	70±40
84 hr after last dose	50±10	70±20	70±20	50±20
96 hr after last dose	40±10	50±10	40±20	40±20

The depletion profiles for milk residues were generally consistent, regardless of treatment regime. For the 2- and 8-dose treatments, residues in the milk of mastitic cows were generally lower than in the milk from healthy cows for milk collected through 36 hours after last dosing. Thereafter, the residues for mastitic cows and healthy cows were comparable. The results from the healthy and mastitic cows are compared in Table 21.

Table 21 A comparison of the mean residues of pirlimycin in the milk of healthy and mastitic cows after 2-dose or 8-dose treatment regimes with pirlimycin HCl at 50 mg/quarter into all 4 quarters

Milk Sample	Pirlimycin concentration (µg/kg)			
	Healthy Cows		Mastitic Cows	
	2 Doses	8 Doses	2 Doses	8 Doses
12 hr after last dose	13600±7180	18600±12200	6610±2340	6300±1710
24 hr after last dose	770±860	1890±1800	420±90	650±340
36 hr after last dose	220±230	450±330	200±30	260±80
48 hr after last dose	100±60	160±40	100±20	120±30
60 hr after last dose	50±20	120±50	80±20	90±30
72 hr after last dose	30±20	80±30	60±20	70±30
84 hr after last dose	30±10	80±30	50±10	70±20
96 hr after last dose	20±10	50±20	40±10	40±20

Three non-GLP residue studies were conducted to evaluate the effect of pirlimycin on starter cultures for cheeses, buttermilk/sour cream and yogurt (Hallberg, 1992; Hallberg, 1998a; Hallberg, 1998b). Pirlimycin concentrations tested were 140 to 590 µg/kg (Hallberg, 1992), 40 to 2400 µg/kg (Hallberg, 1998a) and 20 to 1280 µg/kg (Hallberg, 1998b). In all studies, the observed increase in clotting time was less than twice the clotting time for negative control milk. The lower 95% prediction value for average pirlimycin concentrations was 130 µg/kg. The study concluded that milk collected more than 36 hours after treatment would not adversely affect starter cultures. The study also noted that available milk screening assays could adequatedly detect pirlimycin and could be used to protect starter cultures.

METHODS OF ANALYSIS FOR RESIDUES IN TISSUES

Parent pirlimycin is the only significantly biologically active residue identified in milk and tissues and is, therefore, an appropriate marker residue for monitoring residues of pirlimycin in milk and tissues. Methods have been developed for the analysis of pirlimycin in both milk and tissues and are summarized in Table 22. There are two determinative methods for the quantitation of pirlimycin in milk and liver, one based on a microbiological assay (Benner, 1993; Yein, 1989b) and a second based on an instrumental HPLC/TSP/MS assay (Cazers, 1993; Hornish, 1991; Hornish, 1995a, 1995b). A third method for the specific identification and confirmation of parent pirlimycin in milk and liver is based on HPLC/TSP/MS (Hornish, 1995b). In addition, there are three commercially available screening assays for the detection of antibiotic residues in milk that have been tested against pirlimycin to establish the utility of these assays for detecting pirlimycin (Yein, 1992a; Yein, 1992b). These assays are the Delvotest®P (or Delvotest®SP), the *B. stearothemophilus* Disc Assay (BSDA), and the Charm II Test for Macrolide assay (Charm II) (Hornish, 1993a).

Milk

A highly specific mass spectrometric method is used for the simultaneous quantitative (determinative) and qualitative (confirmatory) determination of pirlimycin in milk. A thermospray interface is used to introduce the HPLC effluent into the mass spectrometer. Following chromatographic separation of sample components, a characteristic fragmentation pattern results in 4 principal ions which are detected by selective ion monitoring (protonated molecular pirlimycin, m/z 411). A stereoisomer of pirlimycin serves as an internal reference providing a marker for method recovery and HPLC retention time, and a normalizing ionisation control for the TSP response. A calibration curve is generated by varying the amount of pirlimycin while holding the amount of iso-pirlimycin constant and measuring the ratio of the peak area of the m/z 411 ion response for pirlimycin to iso-pirlimycin. Interference from endogenous matrix components is virtually eliminated by sequential extraction coupled with solid-phase extraction.

Milk samples are fortified with the internal standard, undergo an acidic extraction, are alkalinized and are cleaned up using a solid-phase extraction procedure (SPE). Following evaporation of the SPE product eluant, the final residue sample is re-dissolved for HPLC/TSP/MS analysis.

The method is validated over a range of 50-1200 µg/kg (Table 22, methods 2M and 3M). The method utilizes two concentration ranges (25 µg/kg to 200 µg/kg and from 200 µg/kg to 1200 µg/kg) that result in straight-line linear regression standard curves. The method has a recovery of 85-100% for determination and 100% for confirmation. The limits of quantitation are 50 and 100 µg/kg for the determinative and confirmatory assays, respectively.

The accuracy of the method was examined by analysing five sets of fortified control milk samples at four concentrations ranging from 0 to 800 µg/kg. These samples had been previously analysed by a validated *M. luteus* microbiological

determinative method (Table 22, method 1M). The quantitative analysis was based on the ratio of the peak area responses for pirlimycin to the internal standard for the principal pseudomolecular ion at m/z 411.4. The overall method recovery was 102%. The slope of the concentration added regressed on the concentration found was 1.031, with an intercept at 0.001, and a linear regression coefficient (R^2) of 0.9924.

The precision of the method was judged relative to the bioassay method (Hornish 1991). The day-to-day coefficient of variation (C.V.) of the determination of pirlimycin concentration in the range 200 to 800 µg/kg was ≤ 7%. The within day C.V. of pirlimycin recovery from the spiked samples was ≤ 6%.

The limit of detection (LOD) was estimated from the pirlimycin-free control milk samples in terms of the standard deviation (SD = 0.009) of the quantitative mean at the retention times of the analytes. The estimated LOD for this method based on the quantitative measurements of the m/z 411.4 ion at the appropriate retention times for pirlimycin and the internal standard is 40 µg/kg.

The estimated limit of quantitation (LOQ) was derived statistically where LOQ = quantitative mean + 10 SD. This resulted in an LOQ of 100 µg/kg. However, a subsequent study (Hornish 1995a) led to the revision of this figure down to a validated LOQ of 50 µg/kg, at which point the recovery was 85% and C.V. <8%.

Several parameters were examined to assess method ruggedness. Solid-phase extraction (SPE) column variability was tested by evaluating three lots of SPE columns using triplicate samples of control milk fortified to 400 µg/kg. There was no significant difference between lots. The effect of varying the organic concentration of the SPE elution solution was evaluated and no significant difference was detected (recoveries of 99.6±1.1% and 107.2±13.9%). Evaluation of different times to evaporate the SPE eluant showed that times greater than or equal to 30 minutes gave recoveries significantly different, and it was concluded that samples must not be left for more than 10-15 minutes after dryness is attained. Variability of HPLC columns was examined using different columns and different lots of column packing material. No significant differences were noted.

In the HPLC/MS system, the thermospray vaporiser performance represents the weakest part of the overall method. No performance deterioration (*i.e.*, as evidenced by increased backpressure and increased operating temperatures with subsequent loss of sensitivity and stability of the ion-flux.) attributable to deposition of non-volatile substances in or around the vaporizer orifice were encountered in the development of the HPLC/TSP/MS method.

The procedure typically takes 60 to 75 minutes for 6 samples to be processed to completion. As a result, the stability of the final solution to be analysed on the LC/MS system was evaluated. Several samples that had been analysed within hours of preparation, were re-analysed after 7 days storage at 2-4°C. The results showed that there was minimal loss of sample integrity within the bounds of the variability of the method (<10%). It was therefore concluded that prepared samples could be satisfactorily stored for several days at 4°C if necessary.

Tissues

Liver

The method for the simultaneous determination and confirmation of pirlimycin is based on the HPLC/TSP/MS method described for milk (Hornish, 1992c; Hornish, 1993b; Hornish, 1997c; Hornish, 1998a; Hornish, 1998b).

Liver samples are incubated at 37°C for 24 hours to maximize the reversion of pirlimycin sulfoxide back to parent pirlimycin. The sample is then fortified with the internal standard and undergoes an acidic extraction. The resulting slurry is filtered, the filter cake rinsed, and the combined filtrate partitioned to expel an aqueous phase containing the acid salt of pirlimycin. Additional recovery is obtained by extracting the organic solution with additional water. The combined aqueous solutions are partially evaporated, alkalinized, and further purified by extraction into methylene chloride. This extract is evaporated to dryness and the residue redissolved for HPLC/TSP/MS analysis. The method is validated over a concentration range of 25 to 2000 µg/kg (Table 22, methods 2LI and 3L).

The method was developed originally without the incubation step. Subsequently, it was demonstrated that pirlimycin concentrations increased in samples maintained at room temperature or at 37°C. The original method was validated over a concentration range of 100µg/kg to 1000 µg/kg. An amended method, now referred to as the low range method (Table 22, method 2LL), has been validated over a concentration range of 25µg/kg to 100 µg/kg (Hornish, 1993b). A high range method (Table 22, method 2HL) has been validated for a concentration of 500 to 10000 µg/kg.

Much of the validation work on the HPLC/TSP/MS assay was conducted before the phenomenon of increasing parent pirlimycin was defined and elucidated. The modified method, 2LI, differs from the previously validated methods only in the incubation of the kidney tissue before initiating the extraction/analysis procedure. Therefore, all performance criteria, except recovery and precision, remain the same.

As with the milk method, the HPLC/TSP/MS method is highly specific giving a characteristic fragmentation pattern, detecting 4 principal ions by Selective Ion Monitoring (SIM). Endogenous interference is virtually eliminated by the sequential extraction procedure.

Historically, the method is linear (correlation of (R^2) = 0.990) over the pirlimycin concentration range evaluated, 0 to 1000 µg/kg for 2LL and 2LI. Overall method recovery was 98% in the low and mid range assays, 0 to 500 µg/kg, and 500 to 1000

µg/kg (Hornish, 1997c), and 94% in the high range assay, 1000 to 5000 µg/kg (Hornish, 1997c). For the 2LI method, the mean recovery for incubated samples fortified at concentrations from 540 µg/kg to 2160 µg/kg was 76.4% (Hornish, 1998a).

Precision was evaluated for both the original and revised methods. In the original method, the day-to-day coefficient of variation (C.V.) of the determination of pirlimycin concentration in the range 100 µg/kg to 1000 µg/kg was 7.7%. The within day C.V. of the recovery of pirlimycin from the spiked samples was 5.2%. For the revised (2LI) method, the CV was 8.2% for fortified control samples, but was 12.4% for incurred-residue samples in the concentration range 240 µg/g to 1750 µg/kg (Hornish, 1998a).

The limit of detection (LOD) was estimated statistically (LOQ = mean + 3 SD) from the pirlimycin-free control liver samples. Consequently, the estimated LOD for this method based on the quantitative measurements of the m/z 411.4 ion at the appropriate retention times for pirlimycin and the internal standard is 40 µg/kg. However, during the validation, the operating LOD appeared to be 15 µg/kg (Hornish, 1998a).

The limit of quantitation (LOQ) was derived statistically (LOQ = mean + 10 SD), giving an estimated LOQ of 80 µg/kg (Hornish 1992c). A subsequent study (Hornish, 1998c) led to the revision of this figure down to a validated LOQ of 25 µg/kg, at which concentration the recovery was 85% with a C.V. <8%.

Several parameters were examined to assess the ruggedness of the method. No significant differences were noted based on degree of evaporation of the aqueous sample and there was no detrimental effect noted when the dried residue from the methylene chloride extraction was left for at least 15 minutes under flowing nitrogen and a water bath temperature of ≈70°C. Different HPLC columns and different lots of column packing materials were tested and no significant differences were found.

As noted with the milk method, the thermospray vaporizer performance represents the weakest part of the LC/MS method. No performance deterioration was encountered during the method development.

The extraction procedure (exclusive of the 24-hour incubation) typically takes 60 to 75 minutes for 6 samples to be processed to completion. Several samples that had been analysed within hours of preparation were re-analysed after 12 days storage at 2-4°C. The results showed that there was minimal loss of sample integrity within the bounds of the variability of the method (9.1%), where the ratio of the results at the two time points is not far from 1.0. It was therefore concluded that prepared liver samples could be satisfactorily stored for several days at 4°C if necessary.

Table 22 Analytical methods for the quantitative and confirmatory analysis of pirlimycin residue in milk and tissues

Matrix	Method ID	Method Description	Assay range	Recovery	LOQ	Ref.
Milk	1M	Quantitative Microbiological Cylinder Plate	20-320 µg/kg	95%	20 µg/kg	Yein, 1989b
Milk	2M	Quantitative HPLC/TSP/MS	50-1200 µg/kg	85-100%	50 µg/kg	Hornish, 1991; Hornish 1995a; Cazers, 1993
Milk	3M	Confirmatory HPLC/TSP/MS	≥100µg/kg	100%	100 µg/kg	Hornish, 1991; Hornish, 1995a; Cazers, 1993
Liver	1L	Quantitative Microbiological Cylinder Plate	40-160 µg/kg	78%	40 µg/kg	Yein, 1991
Liver	2LL	Quantitative HPLC/TSP/MS	25-1000 µg/kg	98%	25 µg/kg	Hornish, 1992c; Hornish, 1993b; Hornish, 1997c
Liver	2LH	Quantitative HPLC/TSP/MS	500-10000 µg/kg	94%	500 µg/kg	Hornish, 1997c
Liver	2LI	Quantitative HPLC/TSP/MS	250-2000 µg/kg	76%	250 µg/kg	Hornish, 1998a; Hornish, 1998b
Liver	3L	Confirmatory HPLC/TSP/MS	≥100 µg/kg	100%	100 µg/kg	Hornish, 1992c; Hornish, 1993b; Hornish, 1998b
Kidney	2K	Quantitative HPLC/TSP/MS	25-200 µg/kg	87-97%	50 µg/kg	Hornish, 1996; Hornish, 1998c
Muscle	2Mu	Quantitative HPLC/TSP/MS	25-200 µg/kg	86-97%	50 µg/kg	Hornish, 1996; Hornish, 1998c
Fat	2F	Quantitative HPLC/TSP/MS	25-200 µg/kg	90-100%	50 µg/kg	Roof, 1996; Hornish, 1998c

Kidney, Muscle and Fat:

This method for pirlimycin residue in kidney, muscle and fat also is based on the HPLC/TSP/MS method described for milk and liver (Hornish, 1996; Hornish, 1998b; Roof, 1996). These tissues do not require the incubation step necessary for liver because they contain parent pirlimycin as the principle residue. The tissue sample is fortified with the internal standard and undergoes an acidic extraction. Thereafter, the procedure is identical to the liver method. The operational range for the method is 25 to 2000 µg/kg (Table 22, methods 2K, 2Mu, and 2F).

Validations of the method for parent pirlimycin in kidney, muscle and fat were performed as above for the liver method. The quantitative assays for kidney (2K), muscle (2Mu) and fat (2F) all have an LOQ of 50 µg/kg and an LOD of 25 µg/kg. The confirmatory assay has a limit of confirmation (LOC) of 100 µg/kg.

APPRAISAL

Pirlimycin has not been previously reviewed by the Committee. Pirlimycin hydrochloride is a lincosamide antibiotic with activity against the Gram-positive organisms. It is used to treat mastitis in lactating dairy cattle. The drug is administered as an intramammary infusion at a dose of 50 mg pirlimycin/quarter.

Pirlimycin was found to be metabolized in a qualitatively similar manner in cattle and rats. Two minor metabolites were found in cow urine which were not identified in rat urine. Differences in the fecal metabolic profiles of cows and rats are attributable to gut microfloral deactivation and not animal metabolism. The rat appears to be a suitable species for toxicity testing for pirlimycin and its metabolites.

Radiolabelled residue studies were conducted in cattle at the labelled dose, 50 mg pirlimycin/quarter, and at an exaggerated dose, 200 mg pirlimycin/quarter. In all studies, all four quarters were treated. Residues in milk accounted for approximately half of the administered dose. Urine and feces accounted for approximately 13% and 28% of the administered dose, respectively. Residues in tissues were low, accounting for less than 5% of the administered dose.

Total residues in milk consisted almost entirely of parent drug. The concentration of parent drug in milk corresponds closely with the concentration of microbiologically active drug. Radiolabelled residues in milk deplete rapidly following the last dose.

In radiolabelled tissue residue depletion studies, total residues were highest in liver and were detectable for more than two weeks after dosing. Residues were readily detected in kidney but were approximately 10% of the concentration in liver. Significantly lower concentrations were found in muscle and fat. In liver, pirlimycin sulfoxide was the major residue and unchanged pirlimycin was the minor residue. The microbiological activity of parent pirlimycin is approximate 100 times that of the sulfoxide.

Parent pirlimycin is an appropriate marker residue as it represents the nearly all of the residues in milk and a significant, albeit minor, residue in liver. Pirlimycin also corresponds to the microbiologically active residues of concern.

In unlabelled residue studies, cows were treated at the labelled dose, 50 mg pirlimycin/quarter in all four quarters. In liver samples, an incubation step is added to the tissue extraction procedure to convert pirlimycin sulfoxide back to pirlimycin. Using the HPLC/TSP/MS method, residues are measured. Residues in muscle and fat are low or nondetectable at all sampling times (2 – 28 days after dosing). Residues are detected in kidney samples for the first week with means of 460 µg/kg and 60 µg/kg at 2 and 7 days respectively. Liver residues are present for an extended period of time, ranging from 1690 µg/kg at 2 days withdrawal to 60 µg/kg at 21 days withdrawal. In an extended therapy study, cows were treated for 8 days (*vs.* 2 days for the convention therapy) and liver residues persisted for 42 days withdrawal (mean residue = 42µg/kg at 42 days). In a study evaluating drug depletion in mastitic cows, a variety of treatment regimes were tested. In general, depletion profiles were similar for healthy and mastitic cows. Additionally, the extended therapy regimes did not result in significantly higher liver residues at later sampling times.

Milk residues also were evaluated using the 2-dose and 8-dose treatment regimes. Residues following the 8-dose treatment are consistently higher than the residues resulting from the 2-dose treatment at early time points. However, for samples collected more than 60-72 hours after the final treatments, these differences are small. In the mastitic cow milk residue study, residues of pirlimycin were lower than the residues in healthy cows for the first 36 hours after the last dose. Thereafter, milk residues were comparable for healthy and mastitic cows. After 48 hours, there was no significant difference in residue concentrations between the various treatment regimes.

Studies conducted to evaluate the effect of pirlimycin on starter cultures demonstrate that while clotting time is extended in milk containing pirlimycin, it is less than twice the time for negative control milk. Pirlimycin is unlikely to adversely affect the performance of starter cultures when a discard period of 36 hours or more is observed. Additionally, there are a number of screening tests available to detect pirlimycin in milk and protect starter cultures.

Parent pirlimycin is the only significant microbiologically active residue identified in milk and tissues. Methods are available to detect residues of pirlimycin quantitatively and qualitatively. In addition to a microbiological assay, a highly specific HPLC/TSP/MS method is available to measure residues of pirlimycin in tissues and milk.

For milk, the HPLC/TSP/MS method has a limit of quantification (LOQ) of 50 µg/kg and a limit of confirmation (LOC) of 100 µg/kg. The microbiological assay has an LOQ of 20 µg/kg. Recovery is generally good and the assay range is approximately 20-1200 µg/kg.

The HPLC/TSP/MS method can be used for the detection of residues in liver, kidney, muscle and fat. In liver, an incubation step is incorporated into the sample preparation. The range for quantitative analysis is 25-200 µg/kg (500-10000 µg/kg for the upper concentration range in liver). The LOQ is 50 µg/kg for kidney, muscle and fat and 25 µg/kg for liver (500 µg/kg for the upper concentration range in liver). As with milk, recoveries are good. In liver, the microbiological assay has an LOQ of 40 µg/kg and an assay range of 40-160 µg/kg.

The HPLC/TSP/MS method is suitable for monitoring residues of pirlimycin in milk and tissues but, as the method takes more than an hour to process 6 samples, it is considered only moderately practicable.

The Committee noted that the thermospray interface is no longer readily available. However, the method could be modified to use a currently available mass spectrometry interface.

MAXIMUM RESIDUE LIMITS (MRLS)

In recommending MRLs for pirlimycin, the Committee considered the following factors:

- An ADI of 0-8 µg/kg of body weight was established by the Committee based on a microbiological endpoint. This ADI is equivalent to up to 480 µg for a 60 kg person.
- Liver contains the highest concentration of total residues and is the target tissue for residue monitoring purposes. Pirlimycin is the principle microbiologically active residue in tissues and milk. In milk, pirlimycin accounts for nearly 95% of the total residues. Although pirlimycin sulfoxide represents a higher percentage (57-77%) of the total residues in liver than pirlimycin (22-25%), the microbiological activity of the sulfoxide is approximately 1% of pirlimycin. Therefore, pirlimycin is the marker residue in both tissue and milk.
- A validated HPLC/TSP/MS analytical method was use to measure residues of pirlimycin in milk and tissues in the studies submitted for the Committee's review and would be suitable for monitoring residues for regulatory purposes, but for the limitation noted above.
- Concentrations of pirlimycin below 130 µg/kg had no effect on bacterial starter cultures used in the production of fermented milk products.
- The MRLs recommended for liver and kidney were based on residue data from the unlabelled residue depletion study as determined with the HPLC/TSP/MS method. The MRLs recommended for muscle, fat, and milk are based on twice the LOQ for the analytical method.
- A statistical program developed for JECFA (Arnold, 2003) was used to facilitate the assignment of MRLs.

The Committee recommended permanent MRLs for pirlimycin in cattle of 1000 µg/kg in liver, 400 µg/kg in kidney, 100 µg/kg in muscle and fat, and 100 µg/kg in milk, determined as pirlimycin.

The MRLs recommended would result in a theoretical maximum daily intake of 305 µg or 64% of the ADI, based on the model daily food intake of 300 g muscle, 100 g liver, 50 g each of kidney and fat, and 1.5 kg of milk.

Tissue	MRL	Food Basket	TMDI
Muscle	100 µg/kg	0.3 kg	30 µg
Liver	1000 µg/kg	0.1 kg	100 µg
Kidney	400 µg/kg	0.05 kg	20 µg
Fat	100 µg/kg	0.05 kg	5 µg
Milk	100 µg/kg	1.5 kg	150 µg
TMDI			305 µg

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